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OCULAR FLUID DYNAMICS OF THE DOMESTIC FOWL

BY

© JAMES E. BOYD

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Ocular Fluid Dynamics of the Domestic Fowl" submitted by James E. Boyd, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

The domestic chicken (*Gallus domesticus*) develops extreme eye enlargement when reared from hatching in continuous light (24L/0D). The enlargement is associated with a number of eye lesions including reduced corneal height and curvature, reduced outflow facility (C), and elevated intraocular pressure (IOP). This condition has been called light-induced avian glaucoma.

The eye enlargement and associated lesions appeared to be altered only slightly when the chicken was exposed to 24L/0D for the first time at twenty-five days of age. Outflow facility in the normal chicken eye (exposed to a schedule of fourteen hour light and ten hour darkness per day--14L/10D) increased from 1.26 ± 0.23 µl/min/mm Hg at four weeks to 2.18 ± 0.29 at fourteen weeks of age. After exposure to 24L/0D for ten weeks (thirteen and one-half weeks of age) C was decreased significantly ($P < 0.05$) to 1.17 ± 0.21 µl/min/mm Hg. The IOP was reduced from 9.8 ± 0.29 mm Hg to 8.9 ± 0.27 by one week and from 10.6 ± 0.27 to 8.5 ± 0.26 by two weeks of exposure ($P < 0.05$ and 0.01, respectively) and after ten weeks of exposure was 11.8 ± 0.51 mm Hg compared to 12.1 ± 0.34 for control eyes.

The posterior segment was significantly enlarged ($P < 0.01$) by four weeks of exposure to 24L/0D. At twelve weeks of exposure (fifteen and one-half weeks of age), the 24L/0D eyes weighed 4.5 ± 0.19 grams compared to 3.2 ± 0.08 grams for the controls. The enlargement of the chicken eye occurred without elevation of IOP. Although light-induced avian glaucoma is characterized by elevated IOP, this begins much later and apparently does not cause the eye enlargement.

Anterior segment development was greatly retarded in the 24L/OD chicken eye. The volume of the anterior chamber remained about 35 to 40 mm^3 in the 24L/OD eyes while it progressively increased during the growing period to 112.2 ± 4.13 mm^3 in the 14L/10D control eyes at sixteen weeks of age.

The aqueous humor inflow (F), measured by an improved fluorometric technique, increased progressively in normal chicken eyes from 10.1 ± 0.98 µl/min at nine weeks to 21.4 ± 2.68 at fourteen weeks of age. F was significantly reduced to 5.2 ± 0.70 µl/min ($P < 0.05$) by ten days of exposure to 24L/OD (five weeks of age) and remained low thereafter.

It is suggested that reduced aqueous humor inflow in the chicken eye reduces the metabolic supply to the anterior segment, resulting in developmental retardation of the trabeculae and/or drainage mechanism. This may then cause decreased outflow facility and increased IOP in spite of decreased F, but only after prolonged exposure to continuous light.

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1. INTRODUCTION

The domestic fowl (*Gallus domesticus*) develops extreme eye enlargement when reared in continuous light (24L/0D) (Jensen and Matson, 1957). In recent years this light-induced eye enlargement has been the subject of numerous investigations (Lauber, Schutze, and McGinnis, 1961; Lauber, McGinnis, and Boyd, 1965; Lauber and McGinnis, 1966; Lauber, Boyd, and Boyd, 1969a, 1969b, 1969c, 1970; Frankelson, 1969; Smith, Becker, and Podos, 1969). The enlargement is associated with a number of eye lesions, including reduced corneal height and curvature, adhesions of the iris to the lens and/or cornea, reduced outflow facility (C), elevation of intraocular pressure (IOP), damage to the retina and optic nerve, and eventually total blindness. The condition has been called light-induced avian glaucoma and it has some similarities to human open angle glaucoma (Frankelson, Lauber, and Boyd, 1969).

Inhibited eye enlargement in 24L/0D chickens which had been fed Diamox (acetazolamide, a carbonic anhydrase inhibitor which suppresses aqueous humor secretion and thus lowers IOP) suggested to Lauber *et al.* (1965) that elevated IOP may be associated with the eye enlargement found in avian glaucoma. Other studies showed that in 24L/0D chicks C was somewhat higher than normal after four weeks of exposure and became reduced after eight weeks (Lauber *et al.*, 1969b). The early elevated C in these 24L/0D chicks was thought to be a compensatory mechanism to offset a presumed high rate of aqueous humor inflow (F). Preliminary studies by these same authors appeared to show an early

enhancement of F. It was suggested that the eye enlargement in avian glaucoma was due to reduced C, perhaps associated with increased F which caused elevation of IOP and an increase in eye size (Lauber *et al.*, 1969c).

A number of factors appear to contradict this hypothesis of Lauber *et al.* Eye enlargement was significant at four weeks of age in the 24L/OD chick (Lauber *et al.*, 1969c). IOP was not greatly elevated until after twelve weeks of age (Lauber *et al.*, 1969b). The C-value was not reduced until after eight weeks of age (Lauber *et al.*, 1970). Preliminary data suggested that early enhancement of F might explain the lesions of light-induced avian glaucoma, but this has not been confirmed. In fact, continued experience with very young chicks suggests that reliable inflow data cannot be obtained with the apparatus used in this study.

Furthermore, if raised IOP caused eye enlargement as in human congenital glaucoma, then one would expect that both anterior and posterior segments would be enlarged. However, the anterior segment in the light-induced glaucomatous avian eye is actually smaller than that of the normal control eye. The anterior segment of the eye receives a great part of its metabolic supply via the aqueous humor. In the presence of normal F the anterior segment grows normally. Smith *et al.* (1969) showed that Diamox fed to chicks exposed to continuous light caused further reduction of the anterior chamber than did continuous light alone. Because this drug is known to suppress inflow, it can be implied from the data on light-induced avian glaucoma that anterior segment growth is in part regulated by F.

Following from the above implication, it is of crucial importance in the study of avian glaucoma to determine whether or not 24L/0D enhances F. Therefore, studies of the early effect of 24L/0D on F must be conducted. Although the newly hatched chick is too small for these experiments, chicks could be reared in a diurnal light schedule of fourteen hours of light and ten hours of darkness (14L/10D) per day and then transferred to 24L/0D for the first time at some later age. In this way chicks of larger size could be used as experimental subjects for the purpose of obtaining data of aqueous humor inflow during early pathogenesis. Attention should then be directed to the chronological order of development of light-induced changes in the outflow facility, intraocular pressure, aqueous humor inflow, eye enlargement, and dimensional measurements of corneal height and diameter. This course of investigation was followed in the research reported in this thesis.

2. AVIAN OCULAR ANATOMY AND HISTOLOGY

The following is an account of avian ocular anatomy and histology relevant to this thesis. Several texts give a good anatomical and functional description of the avian eye (Walls, 1942; Duke-Elder, 1958; Sturkie, 1965).

2.1 General Features of the Avian Eye

The anterior chamber is well developed in the avian eye but it is usually only the protruding cornea that is visible between the eyelids of the bird, which suggests a small eye to the casual observer. That this is a misconception is readily perceived upon observing the enucleated eye of a bird. The posterior segment is usually much larger in relation to the anterior segment than in the eye of mammals. Between the highly arched cornea and the large vitreous body is an intermediate segment which essentially determines the configuration of the globe. The eye may be termed flat, globular, or tubular, dependent upon the length of the intermediate segment. The chicken eye may be considered globular.

2.2 Cornea

The avian cornea is usually small and highly arched with a nasal eccentricity. The mature chicken cornea may be 2.7 mm high and 10.2 mm in diameter. The cornea is transparent and is composed of closely packed collagen fibrils which are oriented parallel to each other into surface-parallel lamellae. Within intralamellar spaces are fixed corneal cells which by filamentous protoplasmic processes form a syncytium.

These are the living cells of the corneal stroma. The corneal stroma is bounded on front and behind by homogeneous membranes. The front is covered by epithelium and the back is covered by endothelium.

2.3 Sclera

The sclera is the white opaque portion of the eye wall which continues posteriorly from the transparent cornea. In the intermediate segment this opaque tunic contains a ring of flat bony scleral ossicles. Underlying these ossicles and continuing into the posterior segment, a cartilagenous cup is imbedded in the inner half of the scleral tunic. These bony and cartilagenous structures provide strength to the globe wall and help maintain the proper anatomic relationship of the visual components.

2.4 Uvea

2.4.1 Iris

The iris is composed of two heavily pigmented ectodermal layers. The two layers of pigmented epithelium form a light-tight border. In the two layers are the striated sphincter and dilator muscles which control the size of the pupil. There is an intermediate stromal layer to which is closely applied the anterior border layer. Much of the posterior border of the iris rests against the anterior surface of the lens.

2.4.2 Ciliary Body

The ciliary body in birds is divided into two regions, the pars iridis and the pars scleralis.

The pars scleralis contains the striated musculature for accommodative purposes. The pars iridis is composed of many ciliary processes whose secretory epithelium does not differ appreciably from that of mammals (Seaman and Himelfarb, 1963). These ciliary processes constitute the site of aqueous humor production.

2.4.3 Choroid

The abundant ciliary vessels are continuous posteriorly with the choroid which is relatively thicker than in mammalian eyes. The choroidal vessels must be largely responsible for metabolic supply to the retina since birds have little or none of the retinal vasculature found in mammalian eyes. The pecten, a projection from the fundus into the vitreous body, also has been suggested as a source of nutrition for the retinal tissues (O'Rahilly and Meyer, 1961).

2.5 Retina and Vitreous Body

The retina is the innermost layer of the globe tunic. It contains the photoreceptors necessary for vision and a highly developed system of intercommunicating neurons. The retina bounds the interior of the globe which is filled with the vitreous body. The vitreous body is thought to be a gel containing a matrix of collagen fibrils.

2.6 Anterior Chamber

The anterior chamber is bounded anteriorly by the corneal endothelium, laterally by the pectinate ligament and trabecular meshwork, and posteriorly by the anterior surface of the iris and the intra-pupillary part of the anterior surface of the lens. It has the shape

of the corneal dome and contains aqueous humor.

The pectinate ligament (a trabecular ligament), as well as the more delicate trabecular meshwork, is of collagen overlaid by endothelium. The endothelium covering the trabecular elements is continuous anteriorly with that of the back of the cornea and posteriorly gives way to the incomplete endothelium of the anterior surface of the iris.

The anterior chamber angle in the primate eye forms a *cul-de sac* between the root of the iris and the ciliary muscle at the scleral wall. The canal of Schlemm, located in the sclera, is somewhat central to the apex of the angle and constitutes a drainage route through which aqueous humor escapes the anterior chamber. Trabecular sheaths or lamellae of connective tissue covered with endothelium separate the canal of Schlemm from the anterior chamber. These trabecular elements have numerous pores through which aqueous humor must pass to reach the canal of Schlemm. The method of transfer of aqueous humor into the lumen of the canal remains controversial.

In the anterior chamber angle of avian eyes the pectinate ligament and trabecular meshwork separate the anterior chamber from a pair of circumferential venous annuli which usually have an artery located between them. These venous structures are referred to as the ciliary venous sinus by Duke-Elder (1958). These structures serve the same function in the avian eye as the canal of Schlemm in the mammalian eye: by means of intrascleral connections to the episcleral venous system, they serve as routes for the outflow of aqueous humor. To avoid confusion regarding nomenclature, these venous structures are referred to in this thesis as the canal(s) of Schlemm.

3. OCULAR PHYSIOLOGY

Aqueous humor serves as a carrier for nutrients, substrates, and metabolites. The flow and pressure of aqueous humor in the eye depends upon its rate of formation from the blood and on the resistance offered to its passage by the outflow routes. The IOP of the aqueous humor serves to stabilize the dimensions of the globe.

3.1 Measurement of Intraocular Pressure (IOP)

IOP is directly proportional to the flow of aqueous humor as depicted by the formulas:

$$P \propto F$$

$$\therefore P = F \cdot R$$

where P = IOP (mm Hg)

F = inflow (µl/min)

R = resistance to outflow

Thus, the IOP is established by the opposing dynamic processes of inflow and outflow. These two processes appear to be mutually interdependent and are presumably under separate homeostatic control (Weinstein, 1959). The findings of terminal axons or free axon loops in the trabecular meshwork and endothelium of Schlemm's canal appear to support the possibility of a homeostatic reflex controlling outflow (Vrábec, 1954, 1960; Holland, von Sallman, and Collins, 1956).

Davson (1969) has reviewed the methods for IOP measurement. The most accurate measurement of IOP is obtained by the manometric method in which the anterior chamber of the eye is cannulated with

a hollow needle connected to a fluid filled system. In the fluid of the system, pressure is distributed uniformly throughout so a column of fluid may be maintained by the IOP or a pressure transducer can be calibrated to measure the IOP exactly.

Since it is undesirable to cannulate the eyes of human subjects, indirect methods of measuring IOP have been devised. These methods utilize devices termed tonometers which apply pressure against the eye and register the resulting tension in the sclero-corneal coat. The registered value is compared to a standard calibration scale for conversion to mm Hg of IOP (Friedenwald, 1954). IOP measurements with tonometers are estimates, at best, for not everyone is agreed upon the standardization of the calibration scales.

The IOP of the rabbit has been reported to range from 17.6 to 30.0 mm Hg, dependent upon method of measurement (Schmerl and Steinberg, 1946; Guerry, 1951; Langham, 1959b; Sears, 1960b; Kupfer, 1961). Most authors agree that the normal IOP of the rabbit is nearer to 17.6 mm Hg. In the cat the mean IOP was 16.5 mm Hg (Davson, 1969). The IOP of monkeys ranged from 11.0 to 12.9 mm Hg (Bill, 1966b; Sears, 1966; Edwards, Hallman, and Perkins, 1967). Sears (1960a) and Seaman and Himelfarb (1963) found the IOP of chickens to be 15.6 and 18.6 mm Hg, respectively. Smith *et al.* (1969) recorded the IOP in the chicken to be 21.0 mm Hg. A somewhat lower IOP of 13.0 mm Hg was reported for young cockerels by Lauber *et al.* (1970). Possible reasons for these differences are discussed by Lauber *et al.*

Table I presents the IOP values of several experimental animals measured by various authors using several different methods.

Table I. Measured intraocular pressure of several experimental animals. Shown are the IOP values of several animals measured by various authors using different methods of measurement.

Table I

Author	Animal	Method	Mean IOP (mm Hg)
Schmerl and Steinberg (1946)	Rabbit	Schiøtz tonometer	30.0
Guerry (1951)	Rabbit	electromanometer	19.5
Langham (1959b)	Rabbit	electromanometer	20.5
Sears (1960b)	Rabbit	electromanometer	19.0
Kupfer (1961)	Rabbit	electromanometer	17.6
Davson (1969)	Cat	electromanometer	16.5
Bill (1966b)	Monkey	electromanometer	11.0
Sears (1966)	Monkey	electromanometer	12.3
Edwards *et al.* (1967)	Monkey	electromanometer	12.9
Sears (1960a)	Chicken	electromanometer	15.6
Seaman and Himelfarb (1963)	Chicken	Schiøtz tonometer	18.6
Smith *et al.* (1969)	Chicken	Draeger applanation tonometer	21.0
Lauber *et al.* (1970)	Chicken	electromanometer	13.0

3.2 Aqueous Humor Formation

Several mechanisms have been suggested for the formation of aqueous humor: ultrafiltration, diffusion and secretion. Ultrafiltration was first proposed as a mechanism for aqueous humor formation by Leber early in the twentieth century (cited in Adler, 1965). Duke-Elder, Quilliam, and Davson (1940) regarded ultrafiltration as the primary mechanism for aqueous humor formation. Ultrafiltration was thought to result from the differential between capillary pressure and IOP. However, certain diffusible substances such as ascorbic and hyaluronic acids were found to be in higher concentration in the aqueous humor than in the blood. Therefore, in addition to ultrafiltration, some other mechanism must account for aqueous humor formation.

Diffusion (or dialysis) had been proposed as the mechanism responsible for aqueous humor formation (Duke-Elder, 1926; Duke-Elder and Duke-Elder, 1932). Again because of substances in higher concentration in the aqueous humor than in plasma, Duke-Elder (1937) conceded that dialysis alone could not wholly explain the mechanism for aqueous humor formation.

Friedenwald and Stieler (1938) proposed a mechanism of secretion responsible for F. It was thought that the aqueous humor was secreted by the epithelium of the ciliary body. Duke-Elder and Davson (1943, 1948, 1949) provided support for a secretory mechanism for aqueous humor formation. Only by a mechanism which utilizes energy could substances such as ascorbic acid and hyaluronic acid be maintained in the aqueous humor at higher concentrations than in the blood.

It is now accepted that aqueous humor formation results from

a combination of ultrafiltration, diffusion, and secretion (Kinsey and Grant, 1944; Kinsey, 1960; Bárány, 1963; Brubaker and Kupfer, 1966). The rate of aqueous humor inflow has been reported to be pressure dependent (Langham, 1959a; Langham and Eisenlohr, 1963; Bárány, 1963; Bill, 1967; Edwards *et al.*, 1967; Macri, 1967). Since the ultrafiltration component is due to a differential between IOP and a higher capillary pressure, that fraction of aqueous humor inflow should decrease as IOP is elevated (Bill, 1962; Bárány, 1963; Brubaker and Kupfer, 1966; Macri, 1967). The proportion of inflow contributed by ultrafiltration has been estimated to be between 10 and 30 percent in monkeys (Brubaker and Kupfer, 1966; Bill and Bárány, 1966), probably nearer 30 percent (Brubaker, 1970).

The distribution ratio of small ions and nonelectrolytes between aqueous humor and plasma should be unity if diffusion and ultrafiltration alone occurred. However, at physiological pH the proteins confined in plasma are dissociated as anions. To satisfy the Gibbs-Donnan equilibrium, the cations Na^{+}, K^{+}, Mg^{++}, Ca^{++}, etc. would be higher in the plasma than in the aqueous humor. The Gibbs-Donnan equilibrium is satisfied when ions are distributed such that further diffusion toward chemical equilibrium is balanced by the established electrical potential. When aqueous humor is dialyzed against plasma, sodium chloride migrates to the plasma indicating an excess of NaCl in the aqueous humor (Davson, Duke-Elder, and Maurice, 1949). Furthermore, there is an average 10 mV potential between aqueous humor and the plasma, the aqueous humor being positive to the plasma. According to Davson (1969) this potential suggests that none of the ions studied

has been distributed passively. Thus, the Gibbs-Donnan equilibrium does not exist between aqueous humor and plasma. The potential between aqueous humor and plasma is another indication that energy has been used to transfer electrolytes from the plasma to the aqueous humor.

3.3 Aqueous Humor Flow Rate (F)

The aqueous humor flow rate (F) through the eye is a matter of importance concerning the physiology of the aqueous humor. An ideal method for measuring F would entail placing an indicator substance in the aqueous space and permitting dilution of its concentration by newly formed aqueous humor. The rate at which the indicator concentration is decreased could be used to compute F. However, it is practically impossible to place an indicator in the aqueous space without upsetting the homeostasis of the eye.

Several alternative methods have been suggested for measurement or estimation of F. Indirect methods utilizing the coefficient of outflow facility gave a calculated F of 3.5 µl/min in the rabbit (Becker and Constant, 1956). Similarly in humans the calculated F was 3.66 µl/min (Grant, 1950). Computing the volume increase of a clear "bubble" of newly formed aqueous humor as it emerged from the pupil into fluorescein-aqueous humor of the anterior chamber, Holm and Krakau (1968) calculated F in man to be 4.0 to 5.0 µl/min. Dilution of a solution of inulin ^{14}C by newly formed aqueous humor was used by Macri (1967), who estimated F in the cat to be 13.2 µl/min. Utilizing a radioisotope tracer technique, F of the cynomolgus monkey was estimated to be from 1.16 to 2.0 µl/min (Bill and Bárány, 1966; Bill, 1967, 1968). Jones and Maurice (1966), using sodium fluorescein dye applied topically,

found F of the human eye to be 2.5 µl/min. Bárány (1951) injected para-amino-hippuric acid intravenously and estimated F in the mature chicken eye to be about 2 percent of the anterior chamber volume or approximately 2.0 to 2.5 µl/min. Lauber *et al.* (1969a) used intravenously injected sodium fluorescein and estimated F in young cockerels to be about 15 µl/min. Table II shows the inflow of aqueous humor of various experimental subjects measured by various investigators using different techniques.

Sodium fluorescein, the salt of a weak dibasic acid with a molecular weight of about 330, has been used frequently in studies of aqueous humor inflow (e.g. see review by Lugossy, 1959). When illuminated by near ultraviolet light (365 nm), the dye fluoresces strongly in the yellow-green part of the spectrum (525 nm). The tissues of the eye also emit natural fluorescence, similar in wavelength to that of fluorescein, with intensities of emission equivalent to fluorescein concentrations of 2×10^{-8} gram/cc for the cornea and 1×10^{-9} for the iris (Maurice, 1963). To obtain accurate measurements, the concentration of fluorescein in the aqueous space must be well above these concentrations. It is unfortunate that there is no other dye which emits a different wavelength of radiation with an efficiency of fluorescence as great as that of fluorescein. The measured fluorescence of fluorescein in solution has been shown to be proportional to concentration up to 1×10^{-5} gm/cc (Goldmann, 1950; Langham and Wybar, 1954).

The use of a dye indicator for determining F is reliable only if the body's handling of the dye is unbiased, and if the dye can be readily and accurately detected within the expected physiological range. Fluorescein is generally believed to meet these requirements. The

Table II. Inflow of aqueous humor of various experimental subjects measured by various investigators utilizing different techniques.

Table II

Author(s)	Method	Experimental Subject	F-value (µl/min)	Remarks
Grant (1950)	Estimation from C* as determined from IOP changes using electronic Schiøtz tonometer	Man	3.66	Indirect; depends upon C, which varies
Goldmann (1950)	Fluorescein concentration in aqueous humor compared to plasma level	Man	2.0	Somewhat time consuming
Jones and Maurice (1966)	Fluorescein dilution in aqueous humor	Man	2.5	Complicated analysis, time consuming
Holm and Krakau (1968)	Calculated volume change of clear "bubble" of aqueous humor in fluorescein solution of anterior chamber	Man	3.0 - 4.0	Must use miotic drugs to prevent pupillary action
Bill and Bárány (1966)	Analysis of ^{131}I in blood after introduction into anterior chamber via constant pressure infusion	Monkey	1.5 - 2.0	Complicated analysis

* coefficient of outflow facility

cont'd. . . . .

Table II. Continued

Table II (cont'd.)

Author(s)	Method	Experimental Subject	F-value (µl/min)	Remarks
Bill (1967)	Analysis of ^{131}I in blood after introduction into anterior chamber via constant pressure infusion	Monkey	1.16	Complicated analysis
Macri (1967)	Dilution of ^{14}C inulin solution by newly formed aqueous humor	Cat	13.2	Insults homeostatic mechanism with three cannulations
Becker and Constant (1956)	Estimation from C as determined by constant rate infusion	Rabbit	3.5	Indirect; depends upon C, which varies
Sears (1960b)	Estimation from C as determined by constant rate infusion	Rabbit	2.1	Indirect; depends upon C, which varies
Bárány (1950)	Disappearance of PAHA** from aqueous humor	Chicken	2.1 - 2.5	Paracentesis breaks blood-aqueous barrier
Lauber, Boyd and Boyd (1969a)	Fluorescein increase in aqueous humor	Chicken	15 - 20	Offers a simple efficient method (for discussion of validity and precision see Appendix A)

** para-amino-hippuric acid

dye would thus be expected to be a good indicator of inflow by diffusion and ultrafiltration. Furthermore, Frankelson (1969) has shown that the rate of buildup of fluorescein concentration in the anterior chamber of the chicken can be reduced about 50 percent by Diamox. Becker (1954, 1959) had shown that Diamox reduced F in the rabbit 50 to 60 percent and in man 45 to 55 percent. In view of this, Frankelson's finding would suggest that fluorescein is also an indicator of inflow by secretion. Therefore, in experiments in which osmotic and hydrostatic changes are controlled, fluorescein should be reliable as an indicator for aqueous humor inflow by all three mechanisms (Langham and Wood, 1956; Linnèr and Friedenwald, 1957; Householder, Clausen, and Harris, 1965).

Amsler and Huber (1946) used the relationship of illumination intensity to fluorescence extinction as a means of determining the relative fluorescein concentration in the aqueous humor. Goldmann (1950) used a standard beam of activating radiation and visually estimated the fluorescein concentration in the aqueous humor. He used the rate of increase in concentration in the aqueous humor to estimate F. A fluorophotometer has been used to measure the concentration of fluorescein in the aqueous humor (Langham and Wybar, 1954; Maurice, 1963). This device utilizes a slit lamp for illumination of a very small volume of the aqueous space. It is highly sensitive in actual use, but has had certain disadvantages, such as need for manual recording of intensity and for conducting the experiment in a darkened room. Also, great fluctuations of fluorescence intensity because of incomplete mixing of fluorescein in aqueous humor are encountered whan a very small

volume of aqueous humor is monitored for fluorescein. Until only recently a fluorophotometer was not commercially available (Waltman and Kaufman, 1970).

Fluorescein has been introduced into the anterior chamber by diffusion from the conjunctival sac (Weekers and Delmarcelle, 1953) or by iontophoresis across the cornea (Jones and Maurice, 1966) or via the natural inflow routes, from dye introduced intravenously (Lauber *et al.*, 1969a). In the first two of these techniques the fluorescein concentration is decreased as dye is carried out with the normal outflow of aqueous humor. The rate of decrease in fluorescein concentration was used to calculate F. In the third technique fluorescein buildup in the aqueous humor is measured.

To calculate F using the fluorometric technique, it is necessary to know the volume of the aqueous space. Several authors, utilizing different methods, have reported the anterior chamber volume of man to be from 181 to 200 mm^3 (Heim, 1941; Goldmann, 1950; Jones and Maurice, 1963). Lauber *et al.* (1969a) used a geometric technique and determined the anterior chamber volume of the mature chicken eye to be 136 mm^3.

Goldmann (1950) has used fluorescein as an indicator for measuring aqueous inflow and has placed quantitation of F on a firm mathematical basis. One of his formulas may be used when the dye concentration is at peak in the aqueous humor:

$$\frac{C_a}{C_p} = \frac{K_1 + K_2}{K_1 + V}$$

where C_a is the concentration of fluorescein in the aqueous humor, C_p is concentration in plasma. K_1 is a factor for diffusion of fluorescein out into the tissues of the anterior chamber. K_2 is a factor assigned

to concentration of fluorescein entering the anterior chamber from the posterior chamber and is considered to be zero at peak concentration in the aqueous humor. V is that fraction of the anterior chamber volume which flows through the eye per unit time. In humans K_1 is approximately one-tenth of V, therefore Goldmann reasons that the maximum error inherent in the formula is 10 percent.

Goldmann's work demonstrated the validity of using fluorescein in the study of ocular fluid dynamics. Fluorescein concentration in the aqueous humor could be measured accurately without any disturbance to the eye beyond observation of the intensity of fluorescence in the anterior chamber. Complete records of concentration changes with time could be obtained without collecting aqueous humor. The technique used in this thesis follows the principle of Goldmann.

Some of the methods for estimating F are dependent upon assumptions which may not be valid. For example, the outflow facility has been used to estimate F; this value, once assumed to be a constant, is now known to vary. Outflow facility is discussed in greater detail in section 3.4. Other methods involve procedures which upset the intraocular homeostasis, for example, repeated puncture of the cornea or administration of miotic drugs. Others require involved mathematical analysis or utilize somewhat cumbersome recording apparatus such as earlier fluoro-photometers. Most of these techniques are indirect, somewhat awkward to use, and in many cases they are too complicated to be applied routinely to large numbers of experimental subjects. A simple reliable method for determining F in experimental animals as well as in human subjects has not been available. A method which involves minimal

interference with the homeostatic mechanism(s) is required for this purpose. The method outlined by Lauber *et al.* (1969a), improved and used in this study, may be capable of filling this need.

One of the objectives of this study was to improve this fluorometric method for determining F. The procedure is presented fully in the methods and materials section. This method may be considered preferable to most others available at present because:

1. It is a simple routine procedure which yields reproducible results.
2. A continuous record is obtained of fluorescence intensity in the aqueous humor. Conversion of this information to inflow rate is achieved by a simple mathematical procedure.
3. Variations of recorded fluorescence intensity due to convection currents are minimized in this method compared to methods using a slit lamp.
4. Most importantly, interference with the homeostasis of the eye is minimized.

3.4 Coefficient of Outflow Facility (C)

Grant originally assumed that the coefficient of outflow facility (C) was a proportionality constant. He derived it by the following procedure:

$$F \propto P$$

$$\therefore CP = F$$

$$C = \frac{F}{P} = \frac{\mu l/min}{mm\ Hg}$$

where F = flow(μl/min)

P = pressure (mm Hg)

C = constant = coefficient of outflow facility (µl/min/mm Hg)

In most cases F is unknown and an infusion rate of saline solution is substituted. Then the formula for determining C is derived as follows:

$$CP_o = F$$

$$\text{and } CP_i = F + I$$

$$\therefore CP_i - CP_o = (F + I) - F$$

$$\therefore C(P_i - P_o) = I$$

$$C = \frac{I}{P_i - P_o} = \mu l/min/mm\ Hg$$

where P_o = preinfusion pressure (mm Hg)

P_i = pressure induced by infusion (mm Hg)

I = infusion rate = µl/min

The value thus derived is termed the coefficient of outflow facility (C) and is the reciprocal of the resistance offered by the routes of aqueous humor outflow. Although most workers no longer consider C to be a constant, use of the term has gained wide acceptance. In this thesis the conventional C-value will be used in preference to the outflow resistance designation. Grant (1958) suggested that as much as 75 percent of the resistance to outflow occurred at the site of the trabecular meshwork. However, other studies indicate the site of resistance to outflow from the aqueous space is distal to the canal of Schlemm (Swann, 1954; Perkins, 1955; Sears, 1966; Edwards *et al.*, 1967).

Most of the outflow is through the trabecular meshwork of the iridocorneal angle into the canal of Schlemm, thence into the vascular system via the intrascleral veins (Francois, Neetens, and Collette, 1955;

Huggert, Holmberg, and Esklund, 1955). Another portion of outflow, in the mammalian eye, is by means of diffusion into the tissue of the sclera, ciliary body and choroid and eventually into the episcleral venous system (Fowlks and Havener, 1964; Bill, 1966a, 1966b, 1967; Francois, Neetens, Lerroux, and Collette, 1967; McMaster and Macri, 1968). This uveoscleral route has been regarded as an unconventional route of aqueous humor outflow and was estimated to account for about 10 percent of the total outflow of the monkey eye (Bill, 1965).

Since aqueous humor inflow by ultrafiltration is dependent upon a differential between IOP and blood pressure, this factor must be considered when total outflow is estimated by methods in which changes in the IOP are induced without concomitant changes in the blood pressure. As IOP is increased, the rate of aqueous humor inflow is decreased. Several of the methods for measuring C involve inducing IOP increments within the eye. Such measurements would result in an overestimation of C due to reduction of aqueous humor inflow. Reduced rate of flow due to artificially increased IOP is termed pseudofacility (Bárány, 1963; Bill and Bárány, 1966; Brubaker and Kupfer, 1966; Kupfer and Sanderson, 1968; Bill, 1968; Goldmann, 1968). Pseudofacility, depending upon the species, accounts for 11 to 48 percent of the total outflow facility, with a mean value of about 25 percent (Brubaker, 1970).

Several methods have been devised for the measurement of C. Grant (1950) used an indirect tonographic technique and estimated C in the human eye to be 0.24 µl/min/mm Hg. Using the constant rate infusion method (Becker and Constant, 1956; Sears, 1960b), C of the rabbit was determined to be 0.24 to 0.34 µl/min/mm Hg. Bárány (1964) used the constant pressure infusion method and determined C of the

monkey eye to be 0.55 to 0.60 µl/min/mm Hg. Lauber *et al.* (1970) found C of the normal chicken eye to be 2.0 to 2.5 µl/min/mm Hg. Table III shows C values estimated for the eyes of various experimental subjects by investigators using different techniques.

The original assumption that outflow facility is a constant is open to question along several lines. Artifical elevation of IOP lead to the finding that C is pressure dependent, most studies showing that C is decreased as IOP is increased (Langham, 1959b; Armaly, 1960; Bill and Hellsing, 1965; Bill, 1966b; Levene and Hyman, 1969). This raises the possibility that natural variations in IOP may also be accompanied by changes in C. Erickson (1958) noted a diurnal fluctuation in IOP in human subjects associated with a threefold diurnal variation of F. The IOP change was not of the magnitude that would be expected for such a large variation in F. Erickson suggested that for the eye to maintain a relatively constant IOP in the presence of great variations in F, C must be variable.

Table III. C-values estimated for the eyes of various experimental subjects by investigators utilizing different techniques.

Table III

Author(s)	Method	Experimental Subject	C-value µl/min/mm Hg
Grant (1950)	Electronic Schiøtz tonography	Man	0.24
Becker and Constant (1956)	Constant rate infusion	Rabbit	0.35
Sears (1960)	Constant rate infusion	Rabbit	0.24
Bárány (1964)	Constant pressure infusion	Monkey	0.55
Lauber, Boyd and Boyd (1970)	Constant rate infusion	Chicken	2.0 - 2.5

4. METHODS AND MATERIALS

4.1 Experimental Animal and Environmental Conditions

Both the domestic chicken and turkey are known to develop light-induced avian glaucoma. The chicken was the choice of experimental animal because of economy and ease of maintenance. A rapidly growing broiler type chicken, obtained from stock maintained at the University of Alberta Farm for several years, was used in these experiments. Males were used in preference to females. Data was obtained from chicks which were reared from day of hatch under either a diurnal fourteen hour light and ten hour dark schedule (14L/10D) or were reared for 25 days under 14L/10D and then switched to continuous light (24L/0D). The diurnal (14L/10D) photoperiod was selected because it approximated the natural photoperiod of the chicken. The transfer of chicks to 24L/0D at 25 days was to obtain a larger experimental subject for the early stages of pathogenesis of light-induced avian glaucoma.

Water and a commercial food ration (chick starter) were available at all times. The rearing quarters were light-tight cubicles of approximately 80 square feet floor space, with a continuous forced-air ventilation system. Supplemental local heat for the newly hatched chicks was provided by electric brooders which emitted no light. Overhead illumination was supplied by two 100-watt incandescent lamps. The light source of the 14L/10D photoperiod was controlled by a Paragon* poultry time clock. The accuracy of the timer-illumination

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* Paragon Electric Company, Inc., Two Rivers, Wisconsin.

system in providing the desired light environment was monitored periodically by an apparatus previously described (Lauber and Boyd, 1966).

4.2 Eye Enlargement

Eye enlargement was determined using the weight of the eye as a measure. After each measurement for outflow facility, the anterior chamber was allowed to reform, then the experimental eye was enucleated, trimmed of extraocular tissue, and weighed.

4.3 Anterior Chamber Volume

After each measurement of aqueous humor inflow, the experimental eye was enucleated and photographed in front and side views. From the photographs measurements of corneal height and diameter were obtained. These measurements were used for calculating the volume of the anterior chamber according to the procedure described on page 30 in this section.

4.4 Measurement of Intraocular Pressure (IOP)

The IOP was measured by a manometric pressure transducer (Statham p23Dd)* which was connected to the cannula via polyethylene tubing (PE20). The pressure transducer selected has a volume displacement of 0.01 µl per 100 mm Hg, so the movement of fluid required to register a moderate IOP increment is negligible. The pressure transducer signal was led, via appropriate coupler and amplifier, to a Beckman Type R Dynograph recorder**. The output signal of the transducer was calibrated, within the expected range of IOP, against an external water reservoir

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* Statham Instrument Company, Hato Rey, Puerto Rico.

** Beckman Instrument Company, Spinco Division, Palo Alto, Calif.

which could be temporarily connected into the system for this purpose. A Harvard syringe pump (model No. 600-000)* was incorporated into the cannula system on one side. The apparatus is essentially as described by Lauber *et al.* (1970), and is shown diagrammatically in Figure 1 of this report.

For each experiment in which the eyes of chicks were cannulated, a suitable period of time after cannulation (often 50-60 min) was necessary to assure recording of a steady-state IOP.

4.5 Measurement of Aqueous Humor Inflow (F)

The equipment and procedure for F measurement by the fluorescein method used in this study were essentially the same as previously described (Lauber *et al.*, 1969a).

A spectroradiometer**, capable of measuring spectral energy in $\mu w/cm^2$/spectral bandwidth between 380 and 750 nanometers, was used to measure the aqueous humor fluorescence. The spectroradiometer was set to measure spectral intensity at 525 nm, the fluorescence wavelength of fluorescein. The signal output of the spectroradiometer was conducted to a strip chart recorder (Microcord Model 44***) on which a continuous recording of changing fluorescence intensity was obtained.

The light source for exciting fluorescein was a water cooled high pressure mercury arc lamp (500 watt). A Woods filter removed the biologically deleterious (shorter wavelength) ultraviolet radiation

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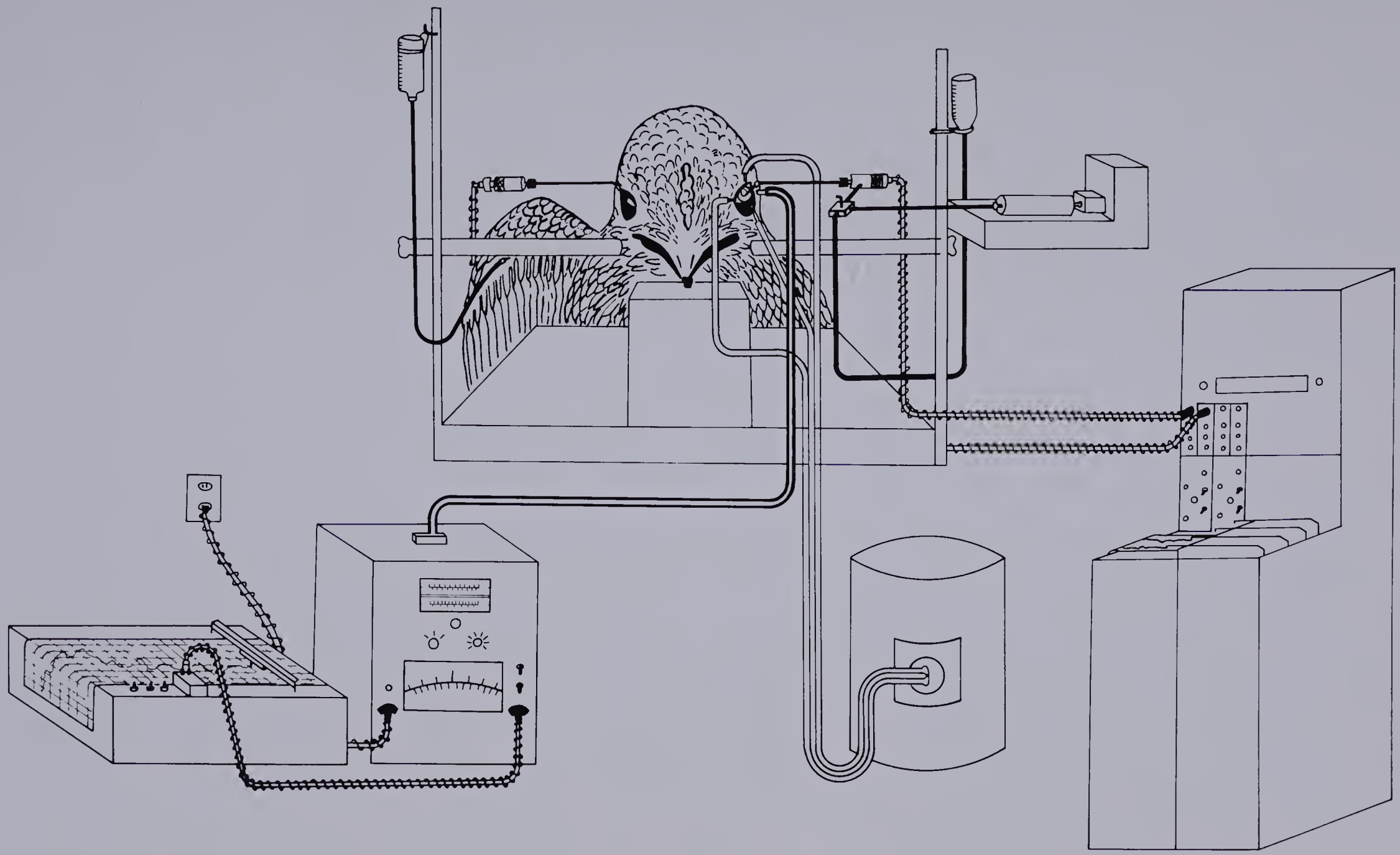
* Harvard Apparatus Company, Dover, Massachusetts.

** Instrumentation Specialities Co., Lincoln, Nebraska.

*** Photovolt Corp., New York, N. Y.

Figure 1. Diagrammatic representation of the experimental set-up for determining intraocular pressure, outflow facility, and rate of aqueous humor inflow. For measuring IOP and C, a cannula in each eye is connected to a pressure transducer whose signal is conducted to a dynograph recorder (lower right). A Harvard syringe pump (upper right) is available for infusion into one eye. For measuring F the fluorescein exciting illumination is conducted to the eye by three fiber optic probes from a mercury arc lamp (lower right-center). The fluorescence in the anterior chamber is directed by a single fiber optic probe to a spectroradiometer (lower left-center) whose signal is conducted to a strip chart recorder where the intensity buildup may be continuously recorded. (From Lauber *et al.*, 1969a).



while transmitting the fluorescein-exciting wavelength of 365 nm. The activating radiation was conducted to the chick's eye by three special glass fiber probes with low refractive index cores which permit the transmission of ultraviolet light. A pickup fiber optic probe was aimed at the pupil to conduct the green light emitted by the excited fluorescein to the spectroradiometer. A plastic probe-holding device maintained the relative positions of illuminating and pickup probes. This device was optimally positioned in front of the cornea (before fluorescein injection) by measuring reflection from the cornea at a maximum illumination at 405 nm, a prominent mercury line in the near ultraviolet.

A solution of 10 percent sodium fluorescein was injected intravenously via an indwelling wing vein cannula at the dosage level of one cc per kilogram body weight, to create a reserve in the blood. The cannula was then connected to a continuous infusion mechanism to deliver additional fluorescein at a rate of 2.5 cc/hr, to assure maintenance of a high level of dye in the blood. After intravenous administration, fluorescein appeared almost instantly in the vessels of the iris. The recording apparatus monitored the increase in fluorescence intensity as the dye entered the aqueous space within the ensuing minutes. The half life of the "nonfluorescein aqueous space" was determined according to the method described by Lauber *et al.* (1969a). In this method the dye-free aqueous humor at the beginning of an experiment was taken as 100 percent "nonfluorescein aqueous space." As the concentration of dye increased in the aqueous humor, the percentage of nonfluorescein aqueous humor decreased and approximated

an exponential decay curve. When plotted against time on semilog graph paper this decay curve yielded a straight line from which the half life was determined. The half life figure was used to estimate the net formation of aqueous humor in the following manner:

$$F = \frac{V}{T^{1}_{\frac{1}{2}}}$$

where F = net inflow (µl/min)

V = volume of aqueous space (mm^3)

$T^{1}_{\frac{1}{2}}$ = half life = minutes required for one complete aqueous volume exchange.

The volume of the aqueous space was estimated by a geometric method based on the assumption that the cornea bounds a segment of a perfect sphere. The corneal height and diameter were measured on front and side view photographs of freshly enucleated eyes. The values of these parameters were introduced into the formula,

$$V = 1/6\pi h(h^2 + 3a^2)$$

where h = corneal height (mm)

a = radius of corneal base (½ corneal diameter--mm)

V = volume of the aqueous space (mm^3).

The length of time required for an experiment was no more than fifteen minutes following injection of the dye. During the course of an experiment the estimated concentration of fluorescein in the aqueous humor ranged from 10^{-6} to 10^{-5} gm/cc. The fluorescence intensity is proportional to concentration in this range (Goldmann, 1950; Langham and Wybar, 1954). The results of inflow measurement by this method are the same as those obtained by Goldmann's formula (page 18), but are obtained more rapidly and simply. The F-values presented

here are presumed to approximate closely the actual values. A discussion of the validity of these results is in Appendix A.

4.6 Measurement of Outflow Facility (C)

The outflow facility of the chick's eye was determined by the constant rate infusion method of Becker and Constant (1953). This method is especially appropriate for use with the chicken eye because the rate of supplemental inflow during infusion is only a very small fraction of the total aqueous humor flowthrough of that eye. Indeed, several successive infusions can be performed throughout an experiment without ever infusing a volume equal to the aqueous space. In other animals such as the cat, approximately one such volume of infusion over a period of time may be required to attain a plateau of IOP.

The coefficient of outflow facility was calculated according to the formula proposed by Becker and Constant (1953),

$$C = \frac{I}{(Pi - Po)}$$

where C = outflow facility (µl/min/mm Hg)

I = infusion rate (µl/min)

Po = steady-state preinfusion IOP (mm Hg)

Pi = plateau pressure during infusion (mm Hg).

During a typical experiment the eye was subjected to three different low rates of infusion (1 to 4 µl/min). Before each succeeding infusion the IOP was permitted to return to preinfusion level. The average of the C-values calculated from these three infusions was taken as the C-value for that eye.

4.7 Plasma Level of Fluorescein

The plasma level of fluorescein during experimental procedures was determined using mature 14L/10D and 24L/0D chickens. Samples of blood were taken at intervals during a period of time which encompassed the time required for a normal experimental procedure. From an indwelling cannula in the wing vein samples were obtained at one-minute and at three successive five-minute intervals following intravenous injection of fluorescein. The fluorescein concentration was determined by comparing the fluorescence intensity in the plasma samples to a standard curve prepared from fluorescence intensities of saline solutions of known concentrations. The results of this procedure are discussed in Appendix B.

4.8 Procedure of C and IOP Experiments

The birds were anaesthetized to a surgical level with Nembutal (sodium pentobarbital) by intramuscular injection into the breast muscle, in dosage of 50 mg/kg body weight. Supplemental anaesthesia was administered similarly if required. The chick was usually wrapped in an envelope of cheesecloth to restrict body and leg movement which could damage the experimental apparatus. The head was held firmly fixed in a device using a set screw applied laterally to each auditory meatus and a notched block of wood into which the bill was inserted.

Following topical anaesthesia with Ophthaine (proparacaine) solution, the anterior chamber of each eye was cannulated with a 27 gauge needle which was connected to a saline filled system. During the process of cannulation the needle shaft was held firmly with a pair of thumb forceps with the bevel facing the corneal surface. Saline

solution was permitted to flow out of the cannula until the bevel was filled by a drop of solution. The eye was then held in a fixed position by gentle pressure with a Storz pick. The cannula was inserted through the cornea from the temporal side to avoid interference with the nictitating membrane. Great care was exercised to prevent the tip of the cannula from touching any surface within the anterior chamber. Intraocular pressure was often quite variable for some time after cannulation before a stable IOP was achieved. After attainment of a stabilized IOP, infusion of saline into the aqueous space caused an elevation of IOP to a plateau with a return to the preinfusion level when infusion was stopped. Usually three successive different low infusion rates were used to induce IOP increments. The average of the C-values determined was taken as the C for that eye.

4.9 Error of Method

Leakage and/or air bubbles in the cannula or tubing were found to completely upset the cannula-pressure transducer system. Physical irritation caused by the cannula invariably resulted in influx of protein into the aqueous humor (plasmoid aqueous humor). Experiments in which any of these factors occurred were considered to be invalid.

5. RESULTS

Chronologically tabulated data of 14L/10D control chickens and of chickens exposed to 24L/OD for the first time at twenty-five days of age are presented in Table IV. Eye weight, C, and IOP were measured and the mean of each was recorded at alternate weeks from four weeks until sixteen weeks of age. The anterior chamber volume and F were measured more frequently during the early stages of pathogenesis. Values expressed in Table IV and in the text are the arithmetic means plus and minus the standard error of each mean. Nearly four hundred chickens were used in these experiments. The number of observations for each mean in Table IV is in parenthesis preceding the mean.

5.1 Eye Enlargement

Eye enlargement occurred within four weeks after exposure to 24L/OD although the IOP was not elevated even at sixteen weeks of age. Figure 2 shows the effect of 24L/OD on eye size when chicks were exposed to the treatment for the first time at twenty-five days of age. The mean weight of the 24L/OD eyes at eight weeks of age (four weeks of exposure to 24L/OD) was significantly increased ($P < 0.01$) when compared to 14L/10D control eyes. The divergence of the mean eye weights of the two groups continued until the experiments were terminated at sixteen weeks of age. The mean weight of the 24L/OD eye at sixteen weeks of age (twelve weeks of exposure to 24L/OD) was 4.45 ± 0.19 grams compared to 3.22 ± 0.08 grams for the 14L/10D control eye. This enlargement of the chicken eye was confined to the posterior segment (Figure 3).

Table IV. Tabulated data of 14L/10D and 24L/0D chickens. The 24L/0D chickens were exposed to the continuous light for the first time at 25 days of age. Eye weight, C-value, and IOP are recorded at alternate weeks. F-values are recorded more frequently, particularly during the first two weeks following exposure to 24L/0D. The number of observations for each entry is in parentheses preceding the mean ± standard error of the mean. Where the difference between a pair of values is statistically significant, a single bar designates $P < 0.05$ and a double bar designates $P < 0.01$.

Table IV

Schedule	Age (Weeks) + Days	Eye Weight (gms)	Anterior Chamber Volume (mm^3)	C-value (µl/min/mm Hg)	F-value (µl/min)	IOP Both Eyes (mm Hg)
14L/10D	4	(5) 1.2±0.02	(9) 27.3±0.81	(7) 1.26±0.23	(10) 9.3±1.05	(26) 9.9±0.29
24L/0D	4	(5) 1.3±0.4	(9) 26.9±0.94	(11) 1.69±0.23	(10) 6.8±0.70	(26) 8.9±0.27
14L/10D	4 + 2d.		(10) 30.6±0.99		(19)12.3±0.96	
24L/0D	4 + 2d.		(10) 29.4±1.05		(20) 8.3±0.63	
14L/10D	4 + 4d.		(9) 33.5±0.76		(29) 9.7±0.83	
24L/0D	4 + 4d.		(9) 29.1±0.98		(29) 8.1±0.75	
14L/10D	5		(9) 34.9±1.00		(19) 8.0±1.02	
24L/0D	5		(9) 32.1±1.65		(19) 5.2±0.70	
14L/10D	6	(5) 1.8±0.04	(9) 41.1±1.23	(12) 1.47±0.19	(10)11.2±1.01	(24)10.6±0.27
24L/0D	6	(5) 1.9±0.02	(9) 31.5±0.84	(11) 1.67±0.24	(10) 5.4±0.74	(25) 8.5±0.26
14L/10D	7		(9) 46.5±2.13		(13) 8.3±1.10	
24L/0D	7		(8) 31.0±0.92		(12) 3.1±0.60	
14L/10D	8	(5) 2.3±0.03	(5) 57.0±2.78	(5) 1.29±0.10	(10) 9.9±1.34	(10)10.8±0.45
24L/0D	8	(5) 2.6±0.04	(5) 39.0±0.84	(5) 1.89±0.23	(12) 4.3±0.54	(10) 9.3±0.62
14L/10D	9		(6) 67.6±3.63		(8)10.1±0.98	
24L/0D	9		(6) 35.6±2.54		(8) 5.0±0.90	

cont'd. . . .

Table IV. Continued

Table IV (Cont'd.)

Light Schedule	Age (Weeks)	Eye Weight (gms)	Anterior Chamber Volume (mm^3)	C-value (µl/min/mm Hg)	F-value (µl/min)	IOP Both Eyes (mm Hg)
14L/10D	10	(5) 2.7±0.13	(9) 69.9±2.49	(12) 2.05±0.20	(9) 12.1±1.50	(24) 10.3±0.36
24L/OD	10	(5) 3.4±0.06	(10) 33.6±1.15	(11) 1.66±0.25	(9) 5.2±0.89	(23) 9.8±0.27
14L/10D	12	(5) 2.7±0.08	(5) 85.4±3.62	(7) 2.05±0.21	(5) 16.6±1.7	(16) 10.8±0.36
24L/OD	12	(5) 3.8±0.14	(5) 38.6±2.84	(6) 2.32±0.22	(7) 2.8±0.35	(16) 9.8±0.28
14L/10D	14	(5) 3.3±0.08	(5)112.1±6.69	(5) 2.18±0.29	(6) 21.4±2.68	(12) 12.0±0.34
24L/OD	14	(5) 4.0±0.16	(5) 57.1±7.50	(5) 1.17±0.21	(8) 6.0±1.54	(10) 11.8±0.51
14L/10D	16	(5) 3.2±0.08	(5)112.2±4.13	(5) 2.35±0.33		(7) 10.5±0.41
24L/OD	16	(5) 4.5±0.19	(5) 36.0±2.15	(5) 1.12±0.28		(9) 10.3±0.62

Figure 2. Effect of 24L/0D on eye enlargement, as measured by weight. Presented are the mean eye weights in grams ± the standard error of the mean of normal (14L/10D) and continuous light (24L/0D) exposed chicken eyes (* designates $P < 0.05$), ** designates $P < 0.01$).

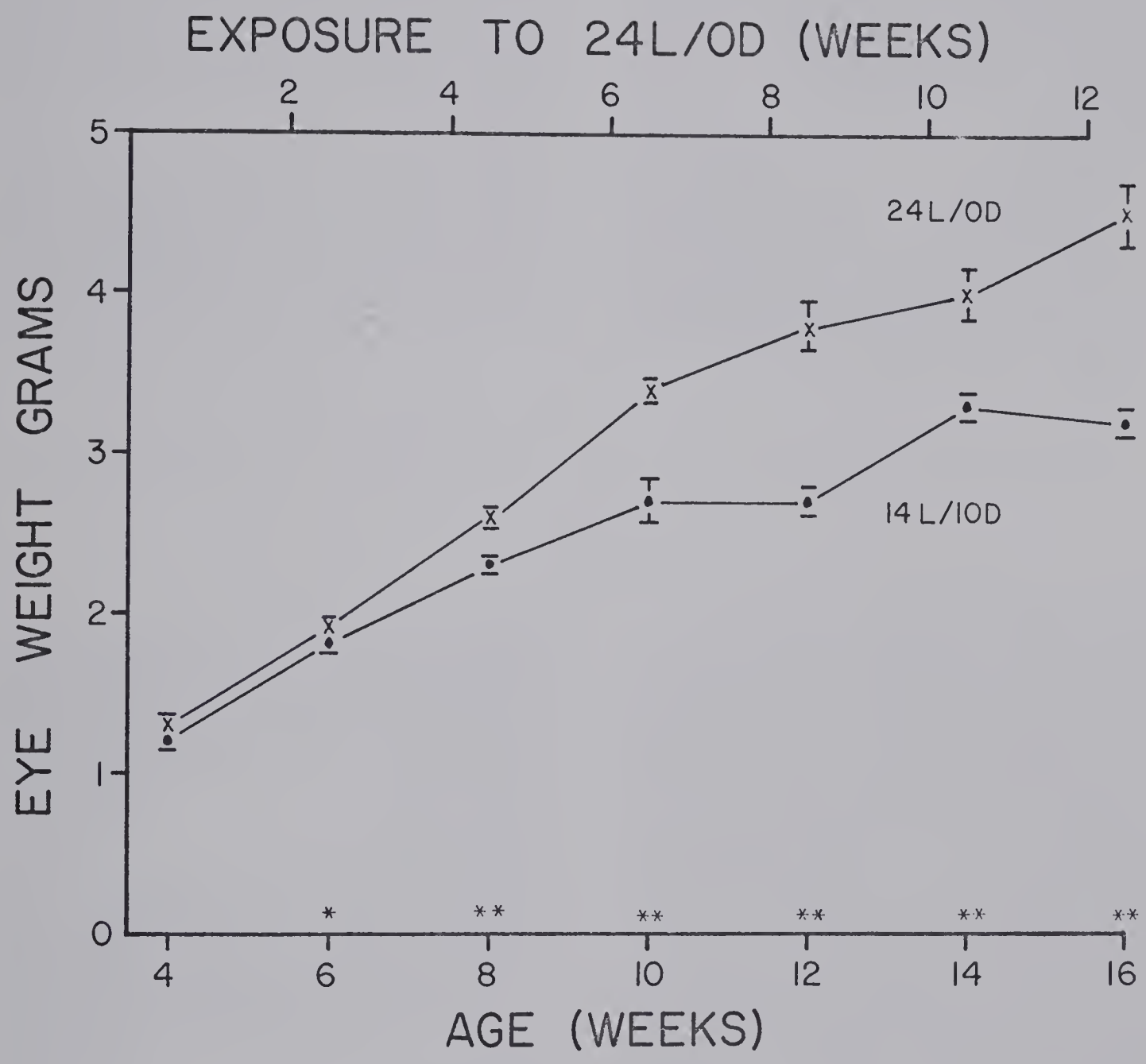
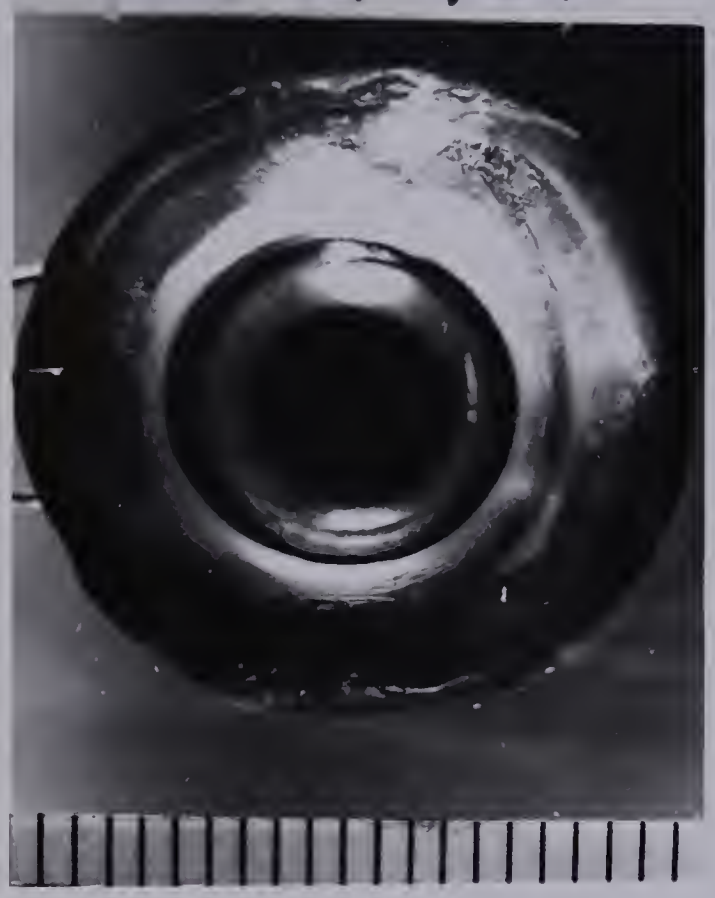
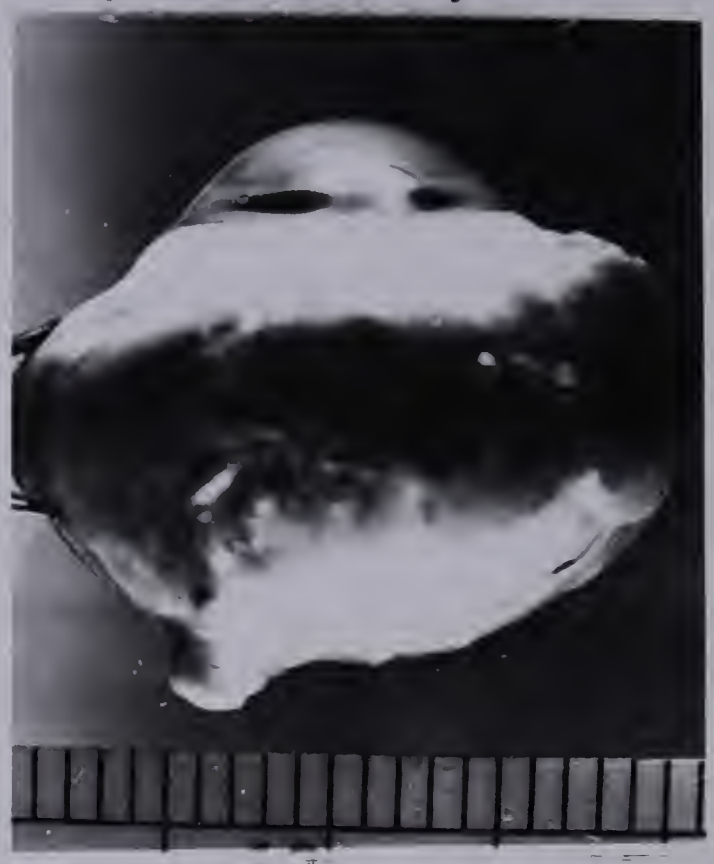

EXPOSURE TO 24L/OD (WEEKS)
2
4
6
8
10
12
24L/OD
14 L/10D
EYE WEIGHT GRAMS
5
4
3
2
1
0
*
**
**
**
**
**
4
6
8
10
12
14
16
AGE (WEEKS)

Figure 3. Photographs of normal (14L/10D) and continuous light (24L/0D) exposed chicken eyes.

CS 16WK
14-2 12/12/69

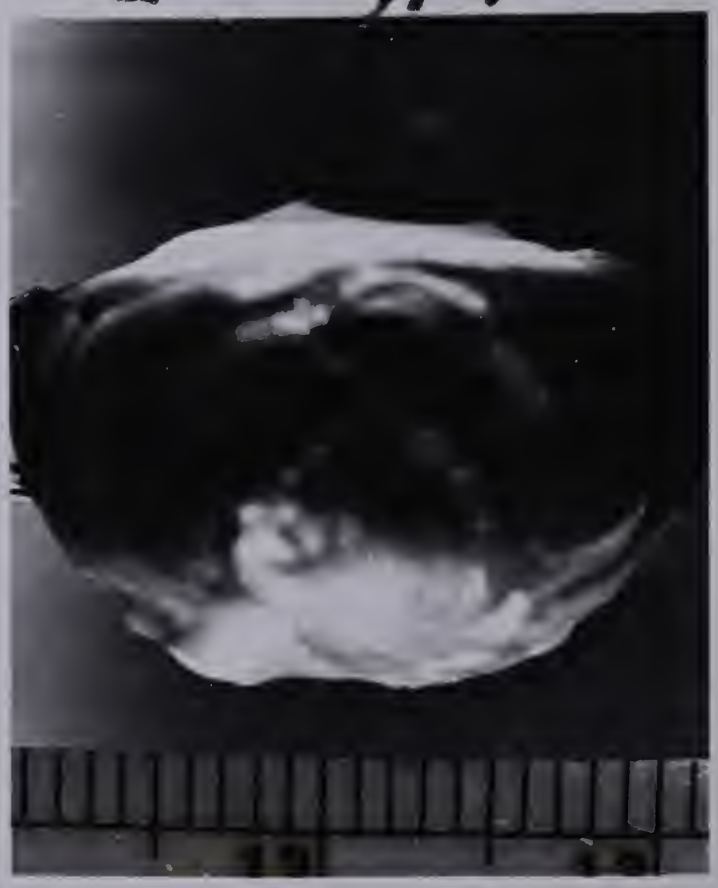


CS 16WK
14-2 12/12/69



CS 16WK
24-1 12/12/69

CS 16WK
24-1 12/12/69



5.2 Anterior Chamber Volume

5.2.1 Normal Birds

The anterior chamber volume of the control 14L/10D chicken eyes increased progressively from 27.3 ± 0.81 mm^3 at four weeks to 112.1 ± 6.69 by fourteen weeks of age (Figure 4). The increase of the anterior chamber volume appeared to be in accordance with the normal growth of the eye.

5.2.2 Continuous Light Birds

In contrast, the volume of the anterior chamber of the 24L/OD eyes increased from 26.9 ± 0.94 mm^3 at four weeks to 39.0 ± 0.84 at eight weeks and remained relatively constant thereafter. The anterior chamber volume indicated a retardation of development of the anterior segment of the 24L/OD eye while eye weight showed that the posterior segment enlarged greatly.

5.3 Aqueous Humor Inflow (F)

5.3.1 Normal Birds

In the young growing 14L/10D chicken, F was in the range of 10.0 µl/min from four to nine weeks of age. After nine weeks of age, F increased progressively to 21.4 ± 2.68 µl/min at fourteen weeks of age (Figure 5).

5.3.2 Continuous Light Birds

During the first week after exposure, the F-values of the 24L/OD chicken eyes were not different from those of the normal 14L/10D eyes. By the tenth day after exposure to 24L/OD, F decreased

Figure 4. Effect of 24L/OD on anterior chamber volume. The anterior segment growth is depicted by the volume of the anterior chamber. Presented are the mean anterior chamber volumes in mm^3 ± the standard error of the mean for normal (14L/10D) and continuous light (24L/OD) exposed chicken eyes (* designates $P < 0.05$, ** designates $P < 0.01$).

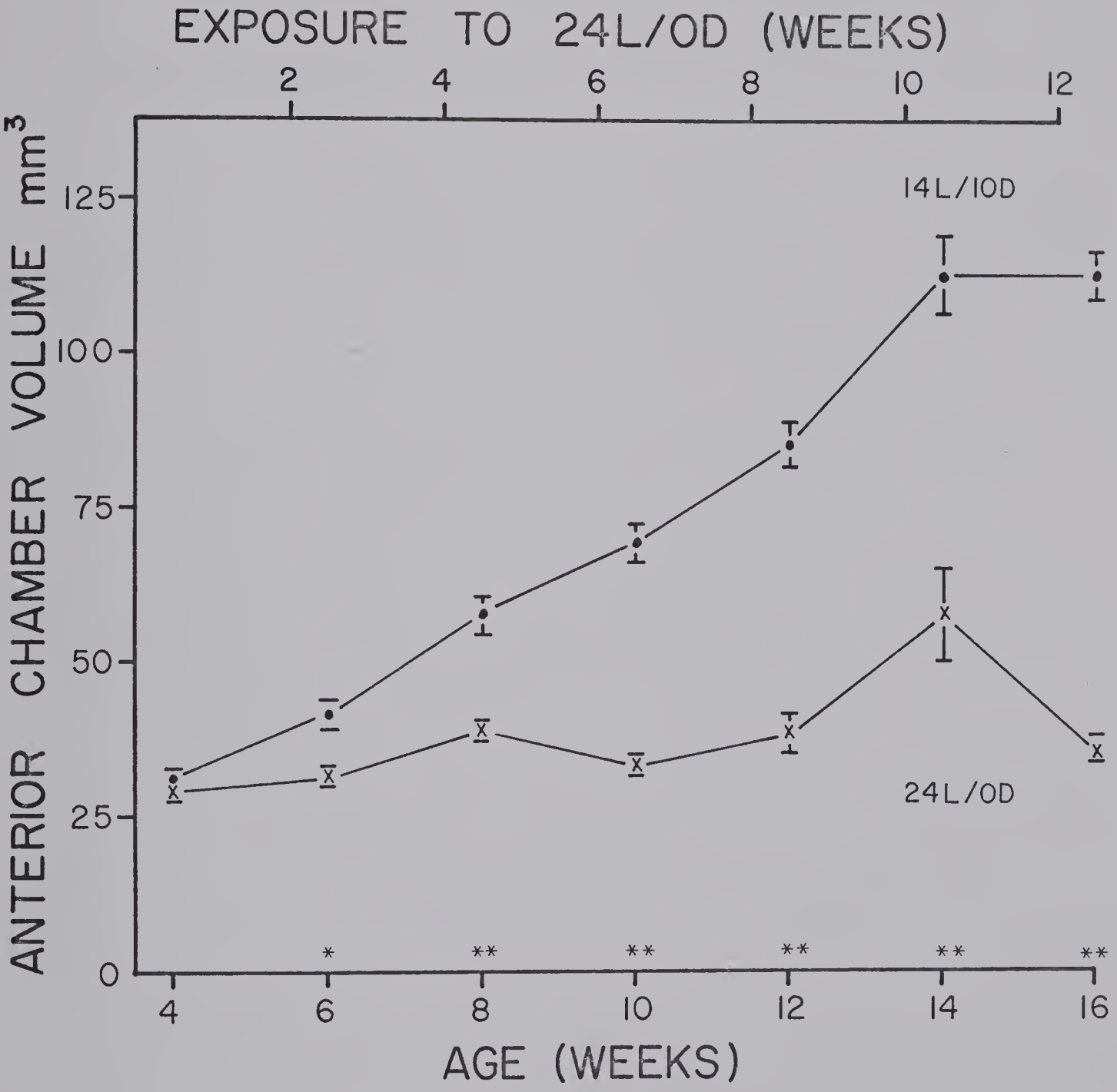
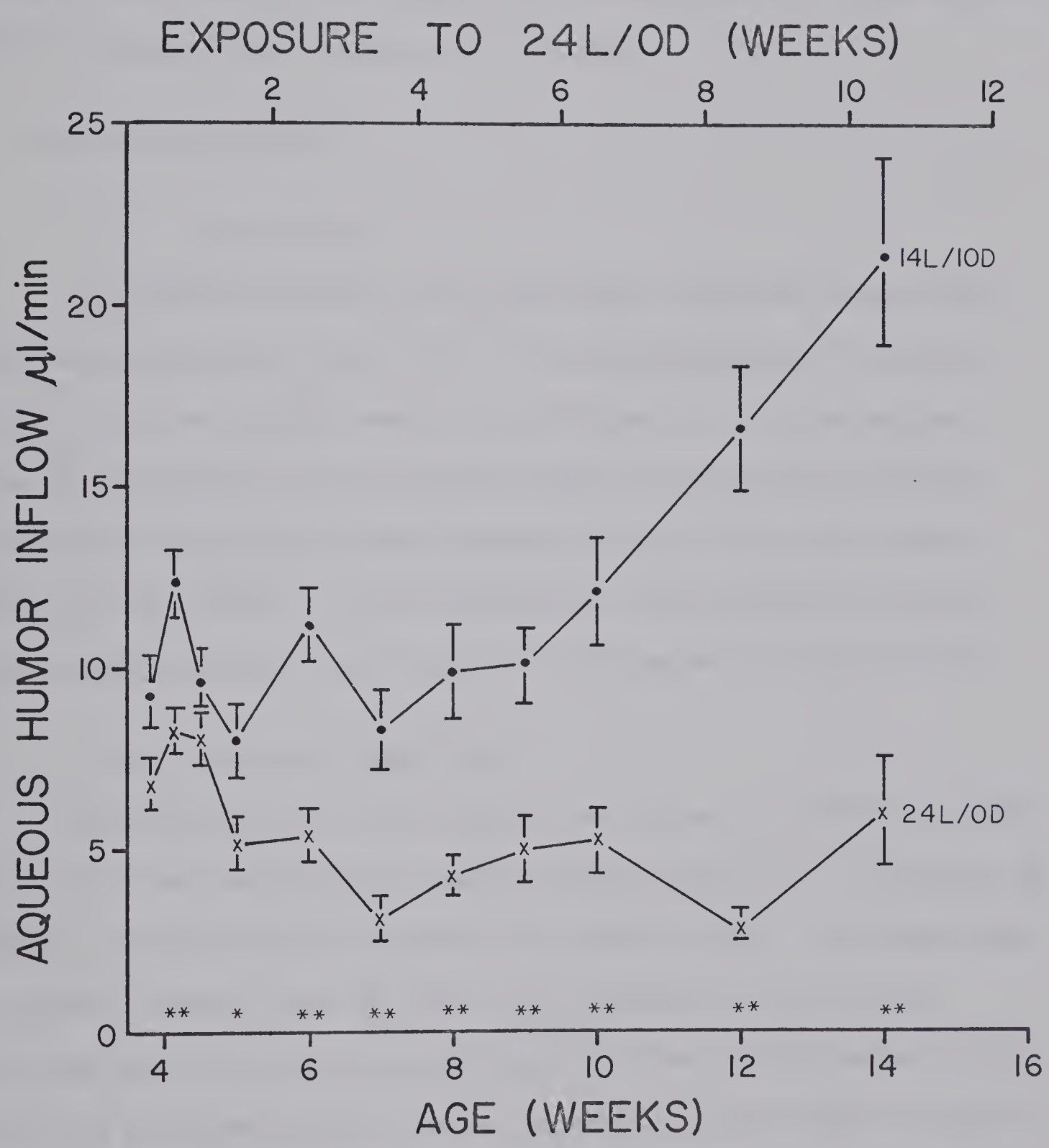

EXPOSURE TO 24L/OD (WEEKS)
2
4
6
8
10
12
ANTERIOR CHAMBER VOLUME mm³
125
100
75
50
25
0
14L/10D
24L/OD
*
**
**
**
**
**
4
6
8
10
12
14
16
AGE (WEEKS)

Figure 5. Effect of 24L/0D on aqueous humor inflow. Presented are the mean rate of aqueous humor inflow in µl/min ± the standard error of the mean of normal (14L/10D) and continuous light (24L/0D) exposed chicken eyes (* designates $P < 0.05$, ** designates $P < 0.01$).


EXPOSURE TO 24L/OD (WEEKS)
2
4
6
8
10
12
25
20
15
10
5
0
AQUEOUS HUMOR INFLOW µl/min
14L/10D
24L/OD
**
*
**
**
**
**
**
**
**
4
6
8
10
12
14
16
AGE (WEEKS)

to 5.2 ± 0.70 µl/min, which was a significant decrease from normal ($P < 0.05$). From five weeks of age (ten days of exposure to 24L/OD) to fourteen weeks of age, F of the 24L/OD eyes remained in the range of 2.8 ± 0.35 to 6.0 ± 1.54 µl/min. The standard error of the mean for F for the entire 24L/OD group was 0.55 µl/min.

5.4 Outflow Facility (C)

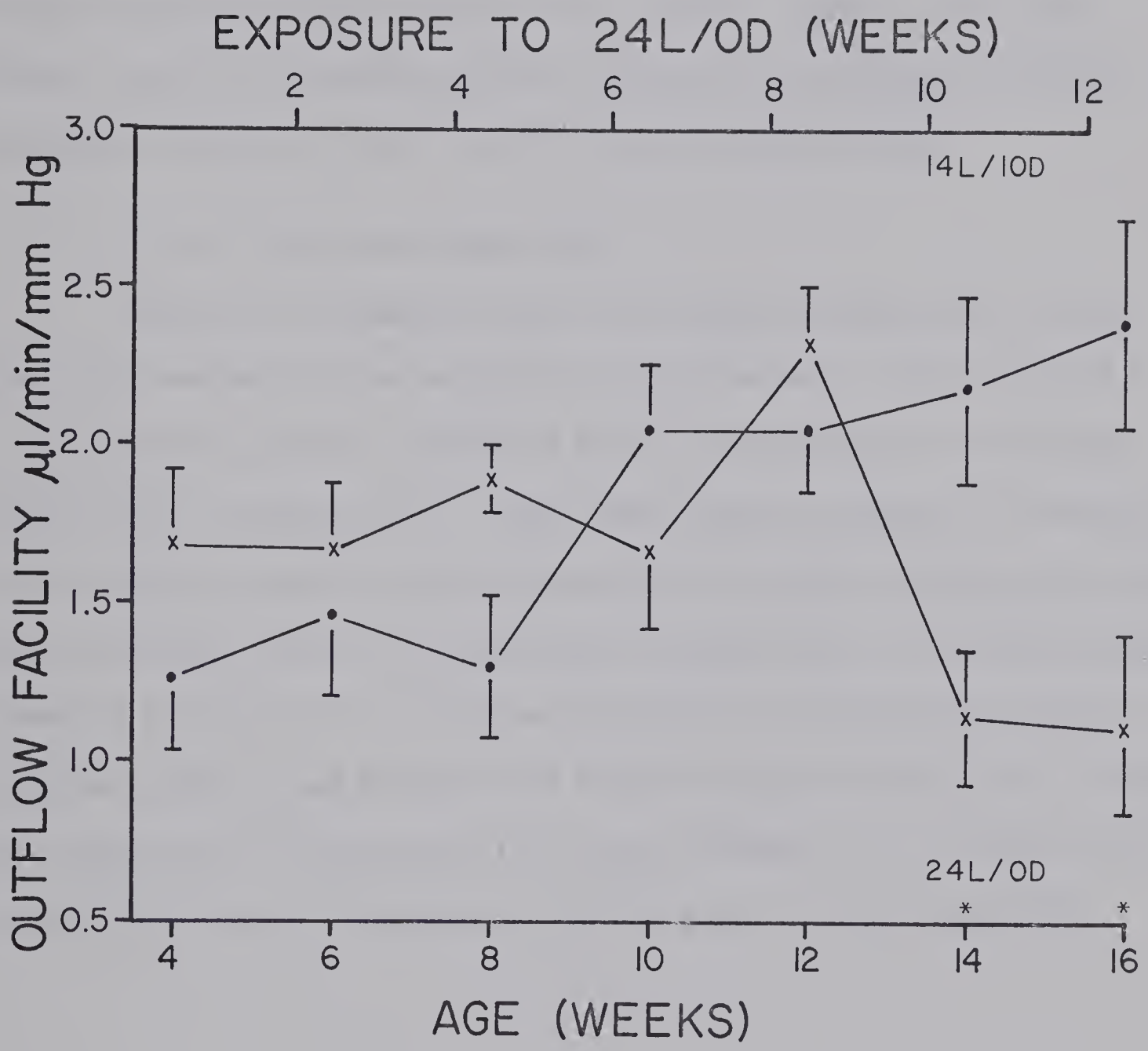
5.4.1 Normal Birds

The outflow facility (C) of the normal (14L/10D) chicken eye increased progressively from 1.26 ± 0.23 µl/min/mm Hg at four weeks to 2.35 ± 0.33 at sixteen weeks of age (Figure 6). The increasing trend of the outflow facility reported here for the growing 14L/10D chicken eye is the same as that reported earlier for similar chicks (Lauber *et al.*, 1970). In the same report, birds exposed to 24L/OD since hatching had very low C values at sixteen weeks (0.16 ± 0.06).

5.4.2 Continuous Light Birds

Outflow facility of the chicken eye exposed to 24L/OD at twenty-five days of age remained at a nearly constant value of 1.7 µl/min/mm Hg for more than six weeks of exposure (ten weeks of age). By eight weeks of exposure (twelve weeks of age) C had increased to 2.18 ± 0.29 µl/min/mm Hg, an increase of more than twice the standard error of the mean (0.25 µl/min/mm Hg) for the entire group. By ten weeks of exposure, C decreased to 1.17 ± 0.21 µl/min/mm Hg, a decrease of more than twice the standard error of the mean for the entire group. The decrease of C was significantly different from normal ($P < 0.05$) for that age group.

Figure 6. Effect of 24L/OD on outflow facility. Presented are the mean outflow facility (C-values) in µl/min/mm Hg ± the standard error of the mean for normal (14L/10D) and continuous light (24L/OD) exposed chicken eyes (* designates $P < 0.05$).


EXPOSURE TO 24L/OD (WEEKS)
2
4
6
8
10
12
14L/10D
3.0
2.5
2.0
1.5
1.0
0.5
OUTFLOW FACILITY µl/min/mm Hg
24L/OD
*
*
4
6
8
10
12
14
16
AGE (WEEKS)

5.5 Intraocular Pressure (IOP)

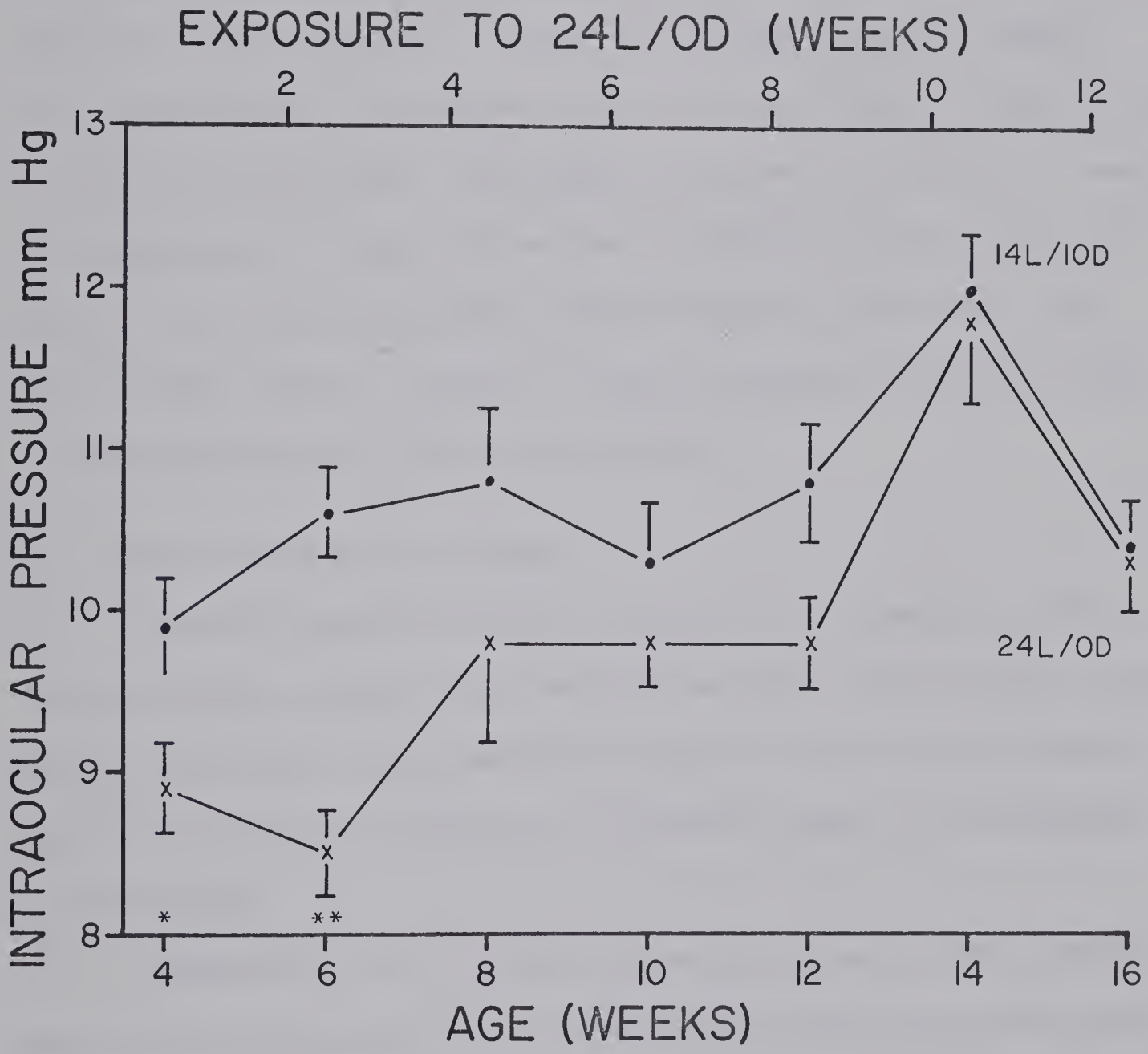
5.5.1 Normal Birds

The intraocular pressure of the 14L/10D eyes was 9.9 ± 0.29 mm Hg at four weeks and 10.5 ± 0.41 at sixteen weeks of age (Figure 7). The IOP in these growing control chickens was similar to that of normal chicks reported earlier (Lauber *et al.*, 1970). Again in that same report, the IOP of chicks exposed to 24L/0D since hatching was pathologically elevated to 31.5 ± 3.81 at sixteen weeks of age.

5.5.2 Continuous Light Birds

The IOP was somewhat lower in the 24L/0D chicken eyes during the first two weeks after exposure to the treatment. The IOP was 8.9 ± 0.27 mm Hg at one week and 8.5 ± 0.26 at three weeks (four and six weeks of age, respectively). These lower values represent a reduction of IOP that was significantly different from control values at the five and one percent levels of significance, respectively. The IOP increased from 9.8 ± 0.28 to 11.8 ± 0.51 mm Hg during the period from twelve to fourteen weeks of age but was 10.3 ± 0.62 mm Hg at sixteen weeks of age. The normal IOP at this age is 12-13 mm Hg (Lauber *et al.*, 1970) so the IOP reported here at fourteen to sixteen weeks of age is not high.

Figure 7. Effect of 24L/OD on intraocular pressure. Presented are the mean values of IOP in mm Hg ± the standard error of the mean for normal (14L/10D) and continuous light (24L/OD) exposed chicken eyes (* designates P < 0.05, ** designates P < 0.01).


EXPOSURE TO 24L/OD (WEEKS)
2
4
6
8
10
12
INTRAOCULAR PRESSURE mm Hg
13
12
11
10
9
8
14L/10D
24L/OD
*
**
4
6
8
10
12
14
16
AGE (WEEKS)

6. DISCUSSION

The domestic chicken develops extreme eye enlargement when reared from hatching in continuous light (24L/0D). The condition has been called light-induced avian glaucoma. The pattern of light-induced changes in chicks exposed to 24L/0D for the first time at twenty-five days has been investigated and compared with that of chicks reared in 24L/0D from hatching. Maintenance of chicks in 14L/10D for twenty-five days after hatching, before their transfer to 24L/0D, does not protect them from glaucomatous lesions induced by continuous light. These changes, however, appear to develop somewhat more slowly than in birds reared under 24L/0D from hatching.

6.1 Light-Induced Avian Glaucoma

Several theories have been invoked in an attempt to explain the pathological changes that characterize light-induced avian glaucoma. Smith *et al.* (1969) suggested that closure of the anterior chamber angle may have caused elevation of IOP which caused eye enlargement in the chicken.

Frankelson *et al.* (1969) offered convincing evidence against angle closure as a mechanism for elevation of IOP and eye enlargement in chickens. He performed iridectomies in young chicks subsequently exposed to 24L/0D. This operation would prevent the iris from blocking the anterior chamber angle (iris bombé). He noted the typical enlargement of the 24L/0D eyes and other characteristic changes of light-induced avian glaucoma, including elevation of IOP by fourteen weeks of age. Angle closure which often causes elevation of IOP in humans

is not the mechanism responsible for eye enlargement found in light-induced avian glaucoma.

Another explanation regarding pathogenesis of light-induced avian glaucoma was presented by Lauber *et al.* (1969c) who suggested increased aqueous humor inflow as a possible cause for elevation of IOP resulting in eye enlargement. The hypothesis suggested that eye enlargement in avian glaucoma was due to reduced outflow facility, perhaps associated with increased aqueous humor inflow, which caused increased IOP and an increase in eye size. The possibility that aqueous humor inflow is increased is supported by the fact that oral Diamox inhibited the enlargement caused by 24L/OD. This drug inhibits the secretory fraction of aqueous humor inflow and thus has a lowering effect on IOP. The C-value in 24L/OD chicks was somewhat elevated after four weeks' exposure, which again suggested an increase in inflow. The high C suggests a compensatory action to offset the assumed high inflow. Preliminary studies seemed to show increased F in chicks exposed to 24L/OD for one week, although this finding was open to some doubt because of the extreme difficulty of making determinations with very young chicks.

Lauber *et al.* (1970) pointed out that eye enlargement was significant in 24L/OD exposed chicks at four weeks of age while IOP was not elevated until sixteen weeks of age. The enlargement was confined to the posterior segment and the anterior segment was much smaller than normal. This situation is unlike that in human congenital glaucoma in which both anterior and posterior segments are enlarged. These factors raise some doubt that increased F is involved in the

eye enlargement of light-induced avian glaucoma.

6.2 Aqueous Humor Inflow (F)

The possibility that continuous light induces an early increase in aqueous humor inflow has been examined. In this study newly hatched chicks were not used because of their small size. Instead, chicks were reared in diurnal conditions and first transferred to 24L/OD at twenty-five days. Early enhancement of aqueous humor inflow was not detected in the eye of the chicken exposed to continuous light. This finding contradicts the increased inflow hypothesis regarding pathogenesis of avian glaucoma. The rate of inflow of 24L/OD eyes was decreased by ten days of exposure and this was presumably responsible for the decreased IOP during the first two weeks of exposure. Inflow of the 24L/OD eyes remained low throughout the ensuing ten weeks after initial exposure to the 24L/OD treatment. The data here provide no explanation for this decrease in F caused by exposure to 24L/OD. A homeostatic control mechanism over aqueous humor secretion has been suggested for monkeys and man (Bill, 1969; Bron, 1969) and for birds by Frankelson (1969) and Lauber *et al.* (1970). Whether such control does indeed occur in the chicken eye, and the mechanism by which continuous light exerts its influence to decrease aqueous humor inflow, must be elucidated with further research.

Since fluorescein was administered to the experimental animals in dosage relative to body weight, it was presumed that a uniform reservoir of dye existed in the plasma that could be acted upon by the inflow mechanism(s) of the eye (see Appendix B). However, a high degree of variability in F between similar experimental birds was

experienced in this study. It is possible that blood pressure differences between animals may be responsible: ultrafiltration, which depends upon hydrostatic pressure, may have contributed to the variation seen among otherwise similar experimental subjects. It is assumed that transport of fluorescein by diffusion and/or secretion would be proportional to the reservoir of free dye in the plasma. In an attempt to minimize blood pressure and other differences among the excitable birds, dosage of anesthetic was carefully adjusted to body weight.

The fluorometric technique for estimating rate of aqueous humor inflow, as outlined in this thesis, is a very simple procedure which may be applied routinely in either clinical or basic research. This technique may be used in conjunction with retinal fluorescein angiography, an established clinical procedure. Studies of the effects of pharmacological agents on ocular fluid dynamics may be conducted routinely and efficiently. With this technique more knowledge has been gained regarding ocular fluid dynamics in the avian eye. Several exciting questions have been raised from the results of this study.

6.3 Outflow Facility (C)

The control mechanism over aqueous humor outflow appears to have lost its effectiveness by ten weeks of exposure to continuous light. The resistance offered by the outflow channels apparently could no longer be lowered by the control mechanism. The increment of IOP in the presence of supplemental inflow (infusion) was larger and yielded a lower C after ten weeks of exposure to 24L/OD.

Outflow facility appears to be decreased as pressure is artifically increased, as stated earlier on page 23. Frankelson (1969) showed

that Diamox injected intravenously in normal (14L/0D) chickens caused a reduction of aqueous humor inflow but only a transitory decrease in IOP. He concluded that C had been decreased to maintain the normal IOP. Certain other physiologically active substances such as norepinephrine and pilocarpine cause an increase in C (Eakins, 1963; Eakins and Ryan, 1964; Sears and Sherk, 1964; Edwards *et al.*, 1967; Hoffman, 1968). These factors would suggest that there is a control mechanism over outflow facility which may be subject to physical and/or humoral stimulation. In avian glaucoma, as F is decreased, the outflow of aqueous humor must be restricted, presumably under the influence of the control mechanism, in an "attempt" to keep the IOP up.

Measurements of C by either tonographic or infusion methods are made under unphysiological conditions at best. In these methods the IOP is elevated abnormally. Since pseudofacility is increased with greater IOP increments, the measured C will be increased with respect to true C. Unless the pseudofacility fraction is known, the measured C is not truly meaningful in absolute terms.

The total measured outflow facility in the normal avian eye may possibly have another component in addition to true facility and pseudofacility. The homeostatic control mechanism over outflow facility in the avian eye may be extremely sensitive and able to exert more effective control than previously suspected. If the outflow facility is increased during infusion, then the computed C would be high. This explanation may account for the somewhat higher C-values found in the chicken eye when compared to those of mammalian eyes. Studies on pseudofacility in the chicken eye are required. Such investigations

may reveal more information regarding homeostatic control over outflow facility. In the chicken (and perhaps the avian eye in general) the sensitive and effective homeostatic control over outflow facility appears to introduce an additional factor to be considered in the measurement of C. The homeostatic control mechanism in the eye presumably is able to detect the very slight increase in IOP when supplemental inflow occurs such as during constant rate infusion. Upon detection of the slight increment of IOP, the control mechanism of the avian eye apparently lowers the restriction to aqueous humor outflow. The net result would be a reduced increment of IOP, and it is this increment that is used for determination of C. Thus C obtained by a method involving artificial elevation of IOP may be falsely high. However, the infusion rates used for determining C in this study represent only a small fraction of normal inflow in the chicken, as contrasted with the comparable situation in mammals. In the cat, for instance, several volumes equivalent to the aqueous space must be infused before a plateau IOP is achieved. The possible source of error as outlined above would thus be minimized in avian studies.

The chicken appears to be an ideal experimental animal for studies on ocular fluid dynamics. The chicken's eye is much larger than casual observation would suggest. This eye apparently has a high rate of aqueous humor flowthrough. Increments of IOP can be induced rather quickly by infusions of saline, at rates of 1 to 4 µl per minute, into the anterior chamber. It is agreed that artificial elevation of IOP is unphysiologic. However, it is suggested that procedures utilized in this study on the chicken eye more closely approximated physiological

conditions than is often the case in experiments with mammalian eyes.

6.4 Anterior Segment Development

The volume of the anterior chamber remained essentially constant in the 24L/OD eyes while in the normal chicken eyes the volume progressively increased with body growth. Outflow facility was not low until ten weeks and IOP was still not elevated at the end of the experimental period, after twelve weeks of exposure to the treatment. The aqueous humor inflow was decreased from ten days after exposure to 24L/OD.

These findings suggest a new hypothesis to explain the phenomenon of light-induced avian glaucoma. It is suggested that reduced aqueous humor inflow causes developmental retardation of the anterior segment. This gradually brings about impaired outflow facility and IOP is elevated secondarily to this, only after prolonged exposure to continuous light. The new hypothesis regarding the development of the anterior segment of the chicken eye is not contradicted by any of the findings in this or previous studies.

Diamox experiments of Smith *et al.* (1969) showed that in 24L/OD the anterior segment of the chicken eye did not develop fully. According to the new hypothesis this could be explained by decreased rate of aqueous humor inflow induced by the drug. Votockŏvă (1962) and Votockŏvă, Praus, Sulcova,Sterbova, and Brettschneider (1966) showed that the arterial supply to the limbal region of the rabbit eye could be interrupted for long intervals without any deleterious effect on the cornea. This suggests that at least a minimal supply of metabolic nutrients may be provided to the cornea by some nonvascular route, presumably the aqueous humor.

This exchange of nutrients is thought to occur primarily in the limbal region. Maurice (1969) showed that in the eye solutes may move through tissues without an accompanying flow of fluid.

The outflow of aqueous humor in the avian eye appears to be almost entirely by way of the canal of Schlemm and intrascleral channels into the episcleral venous system (Vrabec, 1960). This is in contrast to the accessory uveoscleral route of aqueous humor outflow in the mammalian eye. The lack of accessory outflow routes in the avian eye lends support to the new hypothesis presented here on pathogenesis of avian glaucoma. Presumably the outflow channels passing through the sclera become impaired, by retarded development and/or diminished use due to reduced aqueous humor flow, and there is no alternate route to accommodate the outflow. Therefore, in spite of reduced inflow, pathologic elevation of IOP is induced after prolonged exposure to 24L/OD. Further research is necessary to determine the exact mechanisms by which aqueous humor dynamics are altered by exposure to continuous light.

6.5 Posterior Segment Development

The great expansion of the posterior segment of the 24L/OD chicken eye may be only partly attributed to general enlargement in keeping with rapid body growth. One possible mechanism for the excessive expansion of the posterior segment may be similar to that suggested for the swelling of the corneal stroma. Otori has pointed out that an electrostatic association between sodium cations and large polyanions (mucopolysaccharides) is maintained within the corneal stroma (Otori, 1967). Increase in the sodium concentration induces

increased coiling and concentration of the polymer chains (Green, 1969). Apparently the supply of sodium is critical to maintain an optimal amount of sodium bound by the polymer chain. If replacement sodium in the aqueous humor is decreased, the amount of bound sodium decreases and swelling occurs in the corneal stroma due to uncoiling of the polymer chain (Hedbys, 1961). The intermolecular space presumably is filled with water since the dry weight of the enlarged eye was not appreciably greater than normal (Jensen and Matson, 1957).

The vitreous body of the posterior segment contains a complex of protein (collagen) and mucopolysaccharides. Sodium entrance into the chicken eye exposed to continuous light presumably is decreased when aqueous humor inflow is decreased. If this resulted in sodium deficiency in the vitreous the polymer chains would tend to uncoil. Expansion of the posterior segment could thus be induced without an elevation of IOP by the extension of the mucopolysaccharide-protein polymers. Further research on the biochemistry of the vitreous body is necessary to test this hypothesis.

Eye enlargement preceding lowered C and elevated IOP is difficult to understand if the enlargement is to be explained by elevated IOP. The suggested new explanation for globe expansion in the apparent absence of elevated IOP may resolve this contradiction. The hypothesis presented in this thesis regarding anterior segment development offers a solution to another puzzling aspect of light-induced avian glaucoma. The retarded development of the anterior segment may be viewed as a result of impaired nutrition. The findings of this research and the above hypotheses suggest a new way in which to explain light-induced

avian glaucoma and have helped to elucidate the pathogenesis of this interesting disease. At the same time exciting questions have been raised about the more general area of homeostatic control of F and C.

This study has made use of a new fluorometric technique for measuring aqueous humor inflow. The technique offers, for the first time, a simple and efficient procedure for determining one of the most elusive parameters of aqueous fluid dynamics. The avian eye used in this research is exceptionally favorable for ocular studies because of its large size and substantial rate of flowthrough. These new approaches offer great promise for further investigations in both basic and clinical research on the eye.

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APPENDIX A

VALIDITY OF MEASURED F-VALUES

Aqueous humor inflow (F) is composed of three distinct components: diffusion, ultrafiltration, and secretion. The secretory fraction of F may be affected by plasma concentration of solute and blood flow. Ultrafiltration may be affected most by blood pressure since it is dependent upon hydrostatic pressure as a driving force. Diffusion is dependent upon concentration gradient between aqueous humor and plasma.

Assuming that fluorescein is an accurate indicator of aqueous inflow by all three mechanisms (see page 17), it would seem that, if the concentration of fluorescein in the plasma had remained constant, then the calculated rate of aqueous humor inflow would be higher than that reported in this thesis. That would be because the diffusion fraction of F would have contributed more throughout an experiment than it apparently did. However, there was remarkable agreement between F-values determined by the method described in this thesis and those determined by the somewhat more time consuming method and formula of Goldmann (1955). In Goldmann's technique, the percent figure, obtained from the ratio of dye concentration in the aqueous humor at plateau to that in the plasma, is multiplied by the volume of the aqueous space to obtain an aqueous humor inflow rate in µl/min. Table V shows the comparison of results obtained from my data by the two different techniques for measuring aqueous humor inflow in 5 normal (14L/10D) plus 4 glaucomatous (24L/0D) eyes. Figure 8 shows the data obtained by Goldmann's technique plotted against that of the

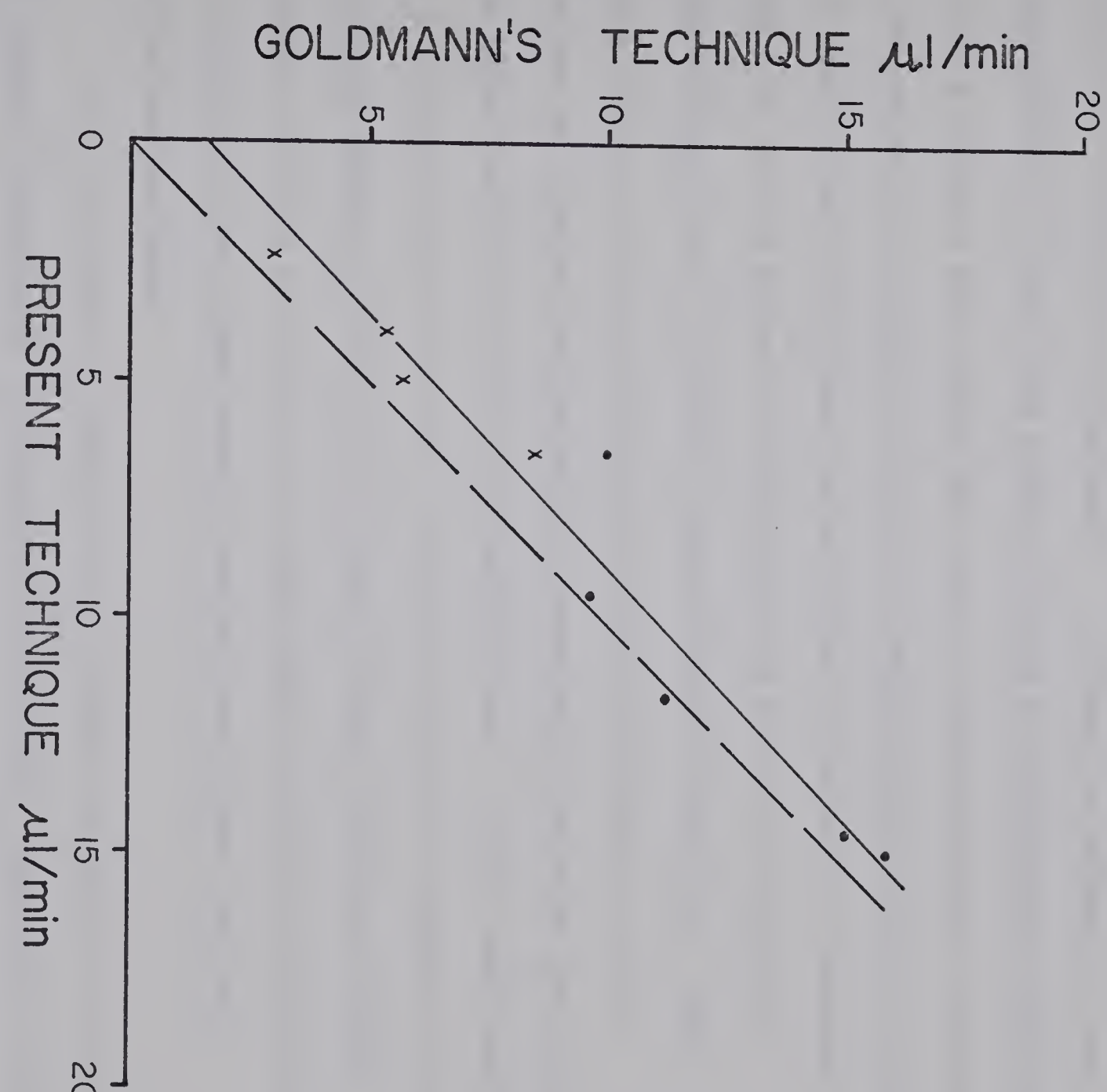
technique used in this report. The degree of correlation was 0.97 ($P < 0.01$). If Goldmann's technique is in fact a valid method of measuring F, then the F-values determined for the chicken eye by the technique described in this report may also be taken to be very good estimates of the actual rate of aqueous humor inflow.

Table V. F-values of the present technique compared to F-value of Goldmann's technique. The present technique utilized the half life ($T\frac{1}{2}$) of the decay of "nonfluorescein aqueous space" in minutes, divided into the anterior chamber volume (Vol. mm^3) to yield the aqueous humor inflow (F-value) in µl/min. Goldmann's technique utilizes the fluorescein concentration in the aqueous humor (at plateau of concentration) [Ca] divided by the fluorescein concentration in the plasma [Cp] to yield a percent figure. That percent of the anterior chamber volume represents the rate of aqueous humor inflow in µl/min.

Table V

	Present Technique			Goldmann's Technique		
	No.	Vol. mm^3	$T\frac{1}{2}$	F-Value	Ca/Cp	%	F-Value
14L/10D	1.	39.9	4.1	9.7	21/88	24	9.6
	2.	39.9	2.7	14.8	34/90	38	15.2
	3.	45.0	3.0	15.0	21/58	36	16.0
	4.	45.0	6.8	6.6	9.9/44	22.5	10.1
	5.	45.0	3.8	11.6	13.5/53	25.5	11.5
24L/0D	1.	30.0	6.0	5.0	20/104	19.2	5.8
	2.	30.0	4.6	6.5	9.8/35	28	8.4
	3.	31.0	14.0	2.2	8.9/88	10	3.1
	4.	31.0	8.0	3.9	9.1/52	17.5	5.4

Figure 8. F-values of Goldmann's technique plotted against F-values of the present technique. The data presented are from 14L/10D (•) and 24L/0D (x) chickens. Each entry represents the measurement of F for a single eye as determined by both techniques. The solid line represents the calculated line of best fit for the data. The broken line represents the line of perfect correlation between data of the two techniques. The correlation coefficient between data of the two techniques was 0.97 ($P < 0.01$).


GOLDMANN'S TECHNIQUE µl/min
0
5
10
15
20
PRESENT TECHNIQUE µl/min
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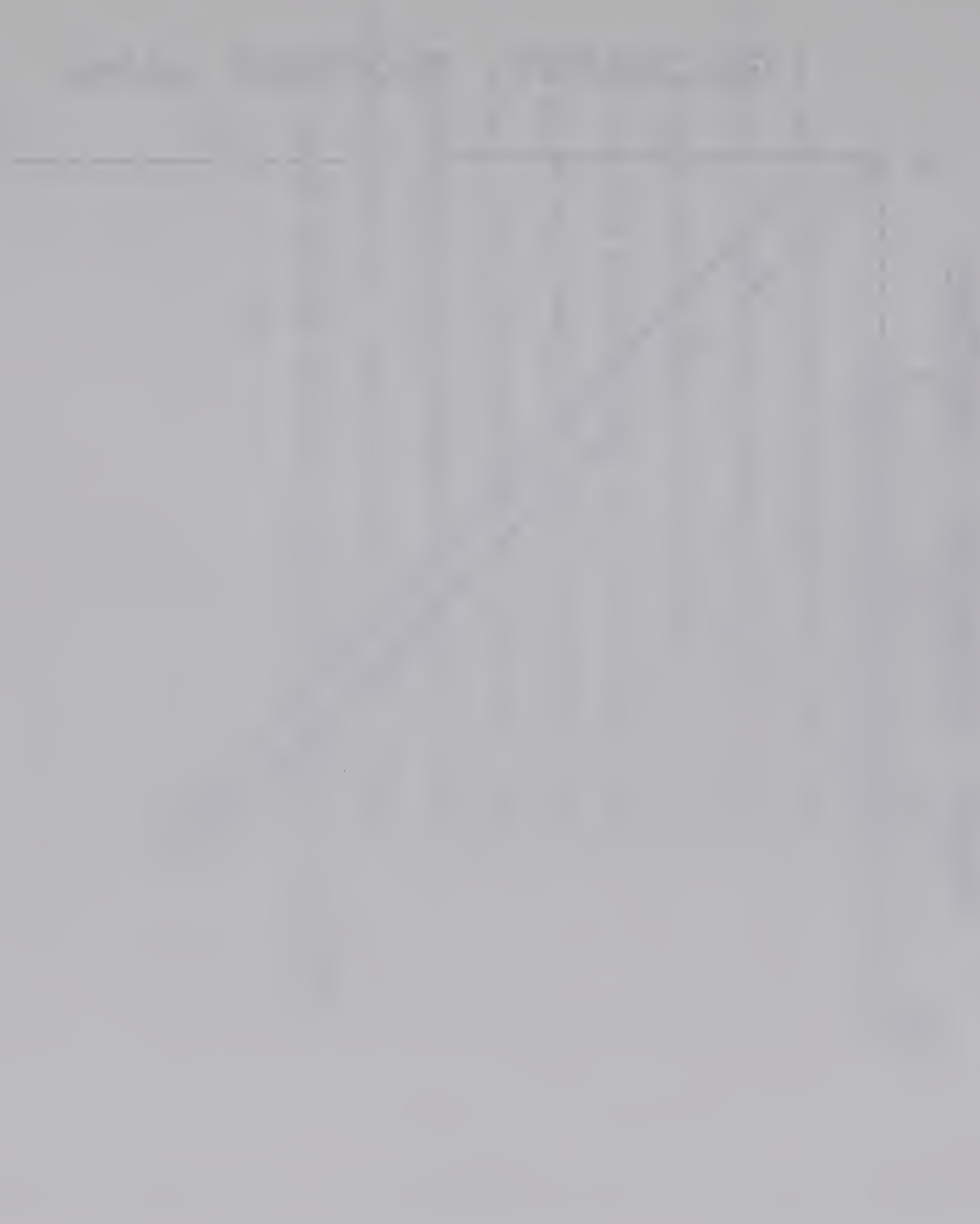
5
10
15
20



APPENDIX B

PLASMA CONCENTRATION OF FLUORESCEIN

It is necessary that a reserve of fluorescein exists in the plasma throughout an experiment. An attempt to maintain a level concentration of dye in the plasma was made by supplementing additional dye at a constant rate during the course of the experimental procedure. It was found that during the course of F-value determinations of chickens (13 weeks of age) the plasma concentration of fluorescein decreased from 0.24 to 0.15 mg/cc in 14L/10D and from 0.24 to 0.17 mg/cc in 24L/0D chickens over a 15-minute period (Tables VI and VII). There was a decrease of about 17 percent in the first 5-minute period. The rate of decrease of fluorescein concentration was much less during the latter two-thirds of the experimental time. In fact, when compared to a straight line of best fit, the decrease from the 5-minute to the 15-minute sample was essentially linear for both 14L/10D and 24L/0D chickens. During the course of an experiment the plasma concentration of fluorescein was always several hundred times higher than that in the aqueous humor.

It can be seen in Tables VI and VII that the degree of variability of plasma concentration of fluorescein in different experimental birds is small. It is apparent that a reservoir of fluorescein existed in the plasma of all experimental birds which was not significantly different (except the 10 min measure $P < 0.05$) between 14L/10D and 24L/0D chickens. This reservoir of fluorescein, although not constant,

could be acted upon by the inflow mechanisms of the eye. Thus, differences between measured F-values is assumed to be attributed to the inflow mechanisms.

Figure 9. Standard curve of fluorescein concentration. This curve was determined by plotting the spectroradiometer measurements of fluorescence intensity against fluorescein concentrations. Each entry represents the mean of five different samples ± standard error of the mean.

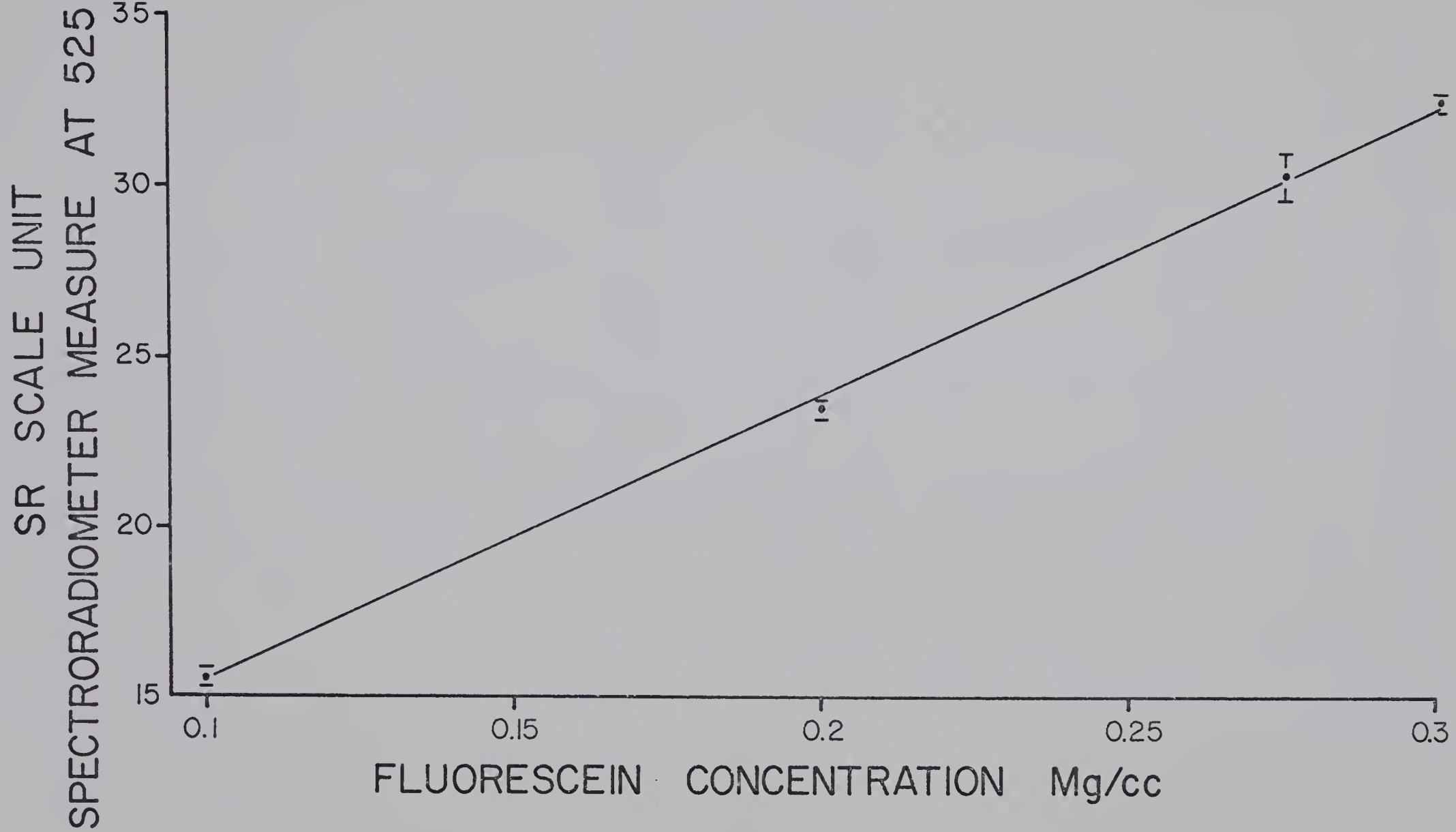

SR SCALE UNIT
SPECTRORADIOMETER MEASURE AT 525 nm
35
30
25
20
15
0.1
0.15
0.2
0.25
0.3
FLUORESCEIN CONCENTRATION Mg/cc

Table VI. Fluorescein concentration in 14L/10D chicken plasma.

The spectroradiometer measurements of the plasma fluorescence intensity are shown for each chicken. The measurements were compared with the standard curve of Figure 1 to obtain the concentration of fluorescein in mg/cc.

Table VI

Spectroradiometer Measurements at 525 nm

	No.	1 min	5 min	10 min	15 min	Body wt. gm
	1	27.75	26.0	21.25	21.0	3080
	2	28.50	23.75	20.25	20.25	2680
	3	25.25	22.25	20.00	19.50	2740
14L/10D	4	28.25	26.25	22.00	22.00	2200
I	5	25.00	19.00	18.00	17.00	2320
	6	27.00	22.50	21.50	21.25	2270
	7	27.25	23.50	19.75	20.00	2280
					Mean body wt.	2510 gm

		Fluorescein Concentration mg/cc			
	1	0.245	0.225	0.167	0.160
	2	0.255	0.198	0.160	0.157
	3	0.217	0.180	0.154	0.147
14L/10D	4	0.252	0.228	0.177	0.177
II	5	0.213	0.142	0.130	0.117
	6	0.237	0.182	0.172	0.167
	7	0.240	0.195	0.150	0.154
	Mean	0.237	0.193	0.159	0.154
	S.E.M.	0.006	0.011	0.006	0.007

Table VII. Fluorescein concentration in 24L/OD chicken plasma. The spectroradiometer measurements of the plasma fluorescence intensity are shown for each chicken. The measurements were compared with the standard curve of Figure 1 to obtain the concentration of fluorescein in mg/cc.

Table VII

Spectroradiometer Measurements at 525 nm

	No.	1 min	5 min	10 min	15 min	Body wt. gm
	1	27.50	24.00	21.25	19.0	2570
	2	27.50	24.00	22.50	22.50	2400
	3	24.75	22.00	20.50	20.00	2560
24L/OD	4	28.25	24.00	22.50	21.50	2575
I	5	26.00	24.00	22.00	21.25	2630
	6	28.50	26.50	24.00	23.00	2420
	7	27.50	22.00	20.75	21.00	2620
	8	28.00	25.50	23.25	23.00	1840
					Mean body wt.	2450 gm

Fluorescein Concentration mg/cc

	1	0.243	0.202	0.167	0.142
	2	0.243	0.202	0.183	0.177
	3	0.210	0.177	0.160	0.154
24L/OD	4	0.252	0.202	0.183	0.172
II	5	0.225	0.202	0.177	0.167
	6	0.255	0.225	0.202	0.190
	7	0.243	0.177	0.163	0.160
	8	0.250	0.220	0.192	0.190
	Mean	0.240	0.201	0.178	0.169
	S.E.M.	0.005	0.006	0.005	0.006